2019

Bioturbation in estuarine sediments: modelling macrofauna-mediated oxygen dynamics

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Bioturbation in estuarine sediments: modelling macrofauna-mediated oxygen dynamics

by

Xiaoyu Fang

A thesis submitted to the University of Plymouth in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Biological and Marine Sciences

September 2019

Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

This research has been conducted under a formal agreement with Ghent University (Belgium), for which a joint award will be awarded.

This study was financed with the aid of a studentship form the Erasmus Mundus and carried out in collaboration with Ghent University.

The following external institutions were visited for consultation purposes:

Publications:

Fang, X., Mestdagh, S., Ysebaert, T., Moens, T., Soetaert, K., Van Colen, C., 2019. Spatio-temporal variation in sediment ecosystem processes and roles of key biota in the Scheldt estuary. Estuar. Coast. Shelf Sci. 222, 21–31.

DOI: https://doi.org/10.1016/j.ecss.2019.04.001

Presentations at conferences:

Fang, X., (2017), “Spatial and temporal variation in bioirrigation and bioturbation and relationships with environmental variables along the estuarine gradient of the Schelde estuary” (Poster), Nereis Park Conference, New York, United States.

Fang, X., (2018), “On the relationships between macrobenthos activities and sediment oxygen dynamics - A case study in the Schelde estuary”, 53rd European Marine Biology Symposium, Ostend, Belgium.

Word count of main body of thesis: 67063

Xiaoju Fang

Signed ……Date……Oct. 15th 2019……

Acknowledgements

The last four years have been the most informative, happiest, thoughtful, altering, moulding, powerful and accepting years of my life. When this PhD journey finally came to an end, I would like to acknowledge the support and encouragement received during my doctoral research.

Back then, I was a beginner sailor who can barely handle a ship steering wheel. However, anything is possible when you have the right people to support you. I have been immensely lucky to have a 'super' supervisory team who guided me through what started as a daunting sea fog, which eventually turned into a sail of intellectual discovery. I am incredibly grateful for all my supervisors who stood by to support me along the way.

Carl, you are the compass. You welcomed me with open arms even though I was lacking the academic background of biology and introduced me those nice and interesting 'macrobenthos' buddies. Over the years, you taught me how to adopt a critical view in marine biology research. You cared so much about my work and responded to my questions and queries so promptly. Your door was always open for me to discuss things. You were by my side throughout this PhD journey, in the climate room, field, onboard, from the first day until the very last stage in finalizing this thesis!

Tony, you are the motive power. You witnessed my 'breakdown' in my toughest time and helped me back on my feet. Thank you for being such a strong and compassionate friend. You helped me to take one step back and see the broad picture when my sail vessel got stuck. Thank you for all the incredible insights into my work and those 'aha' moments!

Tom, you are the water- and wind- proof jacket. Whenever I was freaking out in the storm, you told me it would be fine, and you have been in the same situation yet got it through. Your excellent eye for science has always motivated me to work harder and aim higher.

Thank you for helping me translate the summary to Dutch and backing me up in the last several hours countdown of the finalization!

Karline, you wrote the sailing slogan when our ship set sail. The first time we met, you told me we will make it together, since 'bio-geo-chemistry' corresponds to expertise of all my supervisors. You taught me to use R in modelling and guided me step by step at every crucial moment!

I am still not a skilled sailor, but I learnt how to brave the storms in tough times and enjoy the sea breeze when it is calm. I am confident to compete against my own limits of resilience and brave any challenges in the future. Thank you!

My gratitude goes out as well to the reading and examination committee: Prof. Dr. David Bilton, Prof. Dr. Jan Vanaverbeke, Prof. Dr. Peter Goethals, Prof. Dr. Ann Vanreusel, Dr. Ana Queiros and Dr. Ulrike Braeckman. Thank you for providing me extensive professional guidance and sharing your expertise and knowledge which have honed my thesis. I also want to thank you for letting my pre-defense be an enjoyable moment. It is the most informed, intelligent and inspiring conversation I had about my PhD study!

I am grateful to all of those with whom I have had the pleasure to work with at Ghent University, NIOZ and University of Plymouth. As a marine biologist without great professional skills such as swimming and mud landing, nothing would have been done without all the generous help from my colleagues. Here is an exhaustive list of all the colleagues who helped me out in the field excursions and laboratory work: Sebastiaan Mestdagh, An-Sofie D'Hondt, Tim Tkint, Lisa Mevemkamp, Dirk Van Gansbeke, Bart Beuselinck, Guy Desmet, Mohammed Alsebai, Van Kenhove, Niels Viaene, Elise Toussaint, Emil De Borger, Ninon Mavraki, Ulrike Braeckman, Anna-Maria Vafeiadou, Isolde De Grem, Veerle Rogge, Thomas Kerkhove, Ellen Vlaminck, Lara Macheriotou, the crew of *RV*

Simon Stevin and the students of the IMBRSea programme of UGent. A heartfelt thanks goes to my MARES alumni: Qian Huang, Ee Zin Ong, Valentina Filimonova, Veronica Lo, Sofia Ramalho, Svenja Tidau, Paul Dolder, Cátia Monteiro, Shawn Hinz, Jeffrey Wilson, La Daana Kanhai, who provided support, inspiration, peer pressure, and motivation along this Erasmus Mundus PhD journey.

Francesco, I never expected the co-authorship would turn into a sincere friendship. You have been a role model for me since we met at NIOZ four years ago. I am impressed by your enthusiasm and passion for modelling. You taught me a great deal about research and life. Those fun days in your hometown Lecce, the inspiring modelling brainstorm during the custom-tailored city tour, the discussion about collaboration during ice cream breaks and the R scripts writing in the bar, turned out to be one of the most productive periods in my PhD!

I would like to extend my sincerest thanks and appreciation to my parents for their unconditional support, love and care to an extent that has exceeded all reasonable expectation (no matter what path I choose). We shared our daily life over these years and geographical distance has never been a problem. I inherited the core values 'work hard' and 'love hard' from you. From the bottom of my heart, I thank you. 小花大葱我爱你们! 你们一直是我最大的骄傲!

Benjamin, you are the last piece to solve the Jigsaw puzzle of my life. Eventually, all my 'mad' life choices made sense and the complete picture was revealed: I was meant to take this PhD journey to find my best friend, soulmate and life partner. You were with me in all the ups and downs in this journey and had faith in me even when I felt like digging hole and crawling into one due to frustration and self-doubt. We have been through a lot of tears and joyful moments together and strengthened our commitment and

determination to each other and to live life to the fullest. There are no words to convey how much I appreciate having you in my life!

Dear Mestdagh and de Wilde family, they say you never get to choose your family in-law, but life brought me the best! Thank you for welcoming me warmly into the family and making me experience the sense of belonging in Belgium. I owe special gratitude particularly to Katrien, Karel, Sebastiaan, Rita, Johannes and Nicolas. In those hectic days when the apartment moving, PhD finalization, certificate examination, adaptation to the new job, preparation for the arrival of Chinese family and reception were all scheduled in a few weeks, you were always there supporting Benjamin and me in every possible way and letting us pursue our dreams!

In the pre-defence, I was asked if I would re-do my PhD research in the particular manner given a second chance. To wrap up the reflection on my PhD journey by answering this question: PhD work-wise, there are still some aspects which can be improved (please refer to Chapter 6 General discussion of this thesis); but for my four-year PhD journey, no doubt I would duplicate it without changing a tiny bit, to still have all of you in my life!

I love you all dearly. I dedicate this thesis to all of you.

With much gratitude

Xiaoyu

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Abstract

The aim of this thesis was to provide a comprehensive examination of the roles of macrofauna in estuarine biogeochemistry by adopting a multidisciplinary field and laboratory-based approach and integrating species distribution modelling with biomass-dependent ecosystem function relationships. Eventually, an ecosystem management tool to provide insights into the ecological consequences of anthropogenic disturbance in estuaries was developed using the Western Scheldt as a model system.

To establish a baseline understanding of the spatio-temporal patterns of macrobenthos (activities) and ecosystem functioning in the Scheldt estuary, a seasonal survey was carried out during four consecutive seasons in 2015 and 2016, sampling three habitats with different hydrodynamic regimes (low-dynamic intertidal, high-dynamic intertidal, subtidal) in each of the three main salinity zones (polyhaline, mesohaline, oligohaline). Fluxes of dissolved oxygen, nitrate, nitrite, ammonium and phosphate were measured, as well as environmental properties of the sediment and water, macrofaunal activity (bioturbation and bio-irrigation rates) and macrofauna trait composition with respect to sediment reworking. Luminophores were applied on top of the sediment to measure sediment particle mixing modes and rates. The decline in the concentration of the added inert tracer sodium bromide in the water column was used to estimate pore water exchange rate as a proxy for bio-irrigation. Data and insights obtained from this field survey are presented in **Chapter 2** and **Chapter 3**.

Chapter 2 assesses the spatial and temporal variability of these benthic ecosystem processes (i.e. particle mixing and bio-irrigation) in the Scheldt estuary. Luminophore profiles showed that biodiffusion was the dominant particle reworking mode. Rates of both particle reworking and bio-irrigation were highly variable among seasons and

habitats, and different species were found to contribute unevenly to both processes, and between habitats and seasons. Habitat structuring effects on populations, density-dependent interactions with the habitat, and temperature-driven variability in macrobenthos activity and living position in the sediment are suggested to explain the observed spatial and temporal differences.

In **Chapter 3,** the relative contributions of macrofauna and the environment to the spatial and temporal variability in benthic biogeochemistry were investigated. Using distance-based redundancy analysis, we found total density, bio-irrigation and temperature to be the main contributors to biogeochemical variability, but this model explained only 23 % of the total variation. Variation partitioning and analyses of subsets of the data in the different seasons, showed that processes linked to the biota were lowest and overruled by environmental steering in the coldest period of the year, while processes related the burrowing behaviour of macrofauna, or densities and biomasses of specific sediment reworking functional groups, predominantly determined biogeochemical variability from June onwards. Macrofaunal contribution to biogeochemistry was highest in low-dynamic intertidal habitats that are densely inhabited by biodiffusing and bio-irrigating fauna. We conclude that the impacts of macrofaunal and environmental factors on the biogeochemical fluxes in the Scheldt estuary vary along the estuarine gradients and with season and are thus highly context-dependent. This spatial and temporal variability should therefore be considered to extrapolate biogeochemical fluxes to entire ecosystems.

The impact of two bioturbating benthic invertebrates, *Limecola balthica* and *Hediste diversicolor*, on sediment community oxygen uptake in the Scheldt Estuary was examined in **Chapter 4**. Both *H. diversicolor* and *L. balthica* irrigate the sediment, the former by ventilating their burrows and the latter by siphoning water. Laboratory-controlled

microcosms containing defaunated sediment amended with artificially composed faunal densities of different body sizes were used to test how species identity, habitat, and population density influence O_2 uptake in different habitats (muddy and sandy sediments) in monoculture. Both *L. balthica* and *H. diversicolor* facilitated O_2 fluxes between the sediment and the overlaying water, and a major portion of the variance in sediment metabolism and bio-irrigation could be explained by the per capita body size and density, or by the total biomass of the inhabiting bioturbators. *H. diversicolor* showed a more pronounced relationship between biomass/density and faunal-mediated O_2 consumption than *L. balthica*. Respiration was significant in predicting faunal-mediated O_2 uptake among different species (*H. diversicolor* and *L. balthica*) across different habitats (sandy and muddy sediments); whilst the relationship between bio-irrigation and faunal-mediated O_2 uptake was significant for *H. diversicolor* in both sediment types and and for *L. balthica* in sandy sediments only, with a lower predictive power of bio-irrigation compared to respiration as the predictor. Analysis of covariance demonstrated significant habitat effects in biomass-dependent bio-irrigation, which might be attributed to different physical constraints (e.g. O_2 availability) in sandy and muddy sediments. In summary, we demonstrated that the faunal-mediated O_2 consumption by macrobenthos is density- and biomass-dependent, but the extent to which the variance of faunal-mediated O_2 uptake can be explained is also conditioned by the interplay between the abiotic environment and the biological traits of the species, which is in support of our hypothesis that biological traits (e.g. bio-irrigation) and sediment physico-chemical properties significantly affect faunal-mediated O_2 consumption.

The specific example of using macrobenthos for ecology conservation and estuarine ecosystem management is explored in **Chapter 5**, with *H. diversicolor* as the model species, since this species was found to be the dominant contributor to biogeochemical fluxes in the Scheldt estuary (**Chapter 2-3**). We quantified the contribution of *H.*

diversicolor across a range of biomasses in sediment metabolism and extrapolated the spatial variability of the faunal-mediated O_2 consumption based on its biomass and distribution within its natural habitats in the Western Scheldt. Biomass-scaling of faunal-mediated O_2 uptake by *H. diversicolor* was quantified from laboratory-controlled microcosms containing defaunated sediment with artificially composed faunal densities and body sizes in its habitats (polyhaline sandy sediment, polyhaline muddy sediment and mesohaline muddy sediment) along the estuarine gradients of the Western Scheldt; and the spatial variability of the fauna-mediated O_2 consumption was extrapolated at the landscape scale by combining spatial mapping of *H. diversicolor* developed from multi-quantile regression modelling. Furthermore, the years 1955 and 2010 were compared to investigate changes in *H. diversicolor*-mediated O_2 consumption in relation to the anthropogenic modifications of the estuary between both years. By tuning the quantile of the responses (upper quantile and full quantile), two species distribution scenarios were developed to describe organisms' responses to different environmental constraints. Biomass of *H. diversicolor* was a highly significant (R^2 = 0.9) determinant of fauna-mediated O_2 uptake ($F_{1,26}$ = 119.6, $p < 0.001$) that was unaffected by habitat type ($F_{2, 26}$ = 3.12, p = 0.06). Therefore, the governing function of biomass scaling can be used to scale the biomass-dependent fauna-mediated O_2 uptake to each of three different habitat types. In the intertidal region, maximal fauna-mediated O_2 uptake estimated from the upper quantile regression (tau=0.95) was 8772 and 6201 mol d^{-1} in 1955 and 2010, respectively, corresponding to respective total biomasses of 4407.12 kg and 2915.03 kg *H. diversicolor* for the entire Western Scheldt. The full quantile regression model revealed a decline in total biomass of *H. diversicolor* to 34% of its 1955 level by 2010 (from 3044.71 kg to 1040.03 kg) in the intertidal region of the Western Scheldt, which corresponded with a reduction in the faunal-mediated O_2 consumption by *H. diversicolor* in the intertidal area to 39.7% of the 1955 value by 2010, i.e. a decline from 5401.31 to 2145.93 mol d^{-1}. Overall,

variabilities in maximum current velocity induced by dredging activities most likely affected the spatial distribution and biomass of *H. diversicolor*.

In **Chapter 6**, the key findings from data chapters **Chapter 2-4** are combined to constitute a synthetic discussion about benthos contribution to ecosystem functioning across space and time, along with implications of the modelling methodology applied in **Chapter 5** for the use of macrobenthos for estuarine ecosystem management. The approach adopted in this thesis, which combines small-scale experiments (Chapter 2-4) with broad-scale modelling (Chapter 5), could be used to predict faunal-mediated O_2 consumption at an estuarine landscape level. The integration of species distribution and biomass-dependent ecosystem functioning models allows quantification and upscaling of variation in oxygen dynamics induced by bioturbators at high spatial resolution over a large temporal scale. Additionally, reflections of this study and an outlook for future research are presented.

This thesis bridges small-scale experimental studies with the broad-scale mapping needs of society and managers, developing a predictive framework that can inform policy makers and conservation practitioners for sustainable management of estuaries. This approach is expected to have direct applications for society through the identification of risks to ecosystem functioning and through the generation of advice on species management in view of the provisioning of ecosystem services. It is clear from the work presented in this thesis that macrobenthic bioturbators play an important role in regulating oxygen dynamics in the Scheldt estuary, therefore conservation of specific species (e.g. *H. diversicolor* and *L. balthica*) should be a key priority. These commonly occurring species should be properly monitored and preserved to ensure that their population biomass remains sufficiently high to secure the delivery of ecosystem services. Human activities such as dredging can yield important direct and indirect negative impacts on habitats for macrobenthos, so the natural habitats of key bioturbators should be protected from human impacts through environmental legislation and management (e.g.

European Habitats Directive and the EU Marine Strategy Framework Directive). In a wider context, species and ecosystem conservation and management need to integrate the vulnerability of key species to climate change (e.g. temperature and ocean acidification) and the predicted changes to the provisioning of ecosystem services. Additionally, the potential habitat loss caused by sea level rise and coastal squeeze are major challenges for coastal management in the Scheldt estuary. There is no place for intertidal habitats to migrate along with sea level rise since the estuary is heavily confined by dikes. On the one hand, sea level rise may pump saltwater further upstream; on the other hand, the changes in rainfall may affect freshwater intrusion. Even though more research is needed, the results in this study can act as a baseline for future studies, and the proposed predictive framework is expected to be more broadly applicable to quantify the bioturbation impact of other key species on landscape evolution and ecosystem functionality.

Chapter 1 General introduction

1.1 Macrobenthos in marine soft bottoms

The marine benthic ecosystem is often regarded as the largest ecosystem on Earth and ecosystem processes and functions occurring within it have important effects both locally and globally (Snelgrove, 1999, 1997; Snelgrove et al., 1998). Marine soft sediments are frequently considered as vast and homogenous expanses of mud and sand (Hewitt et al., 2005), in reality these habitats are highly dynamic sedimentary systems characterized by ecological, physical and chemical gradients (McLusky et al., 1993; Meire et al., 2005; Zajac, 2008). Spatial gradients can occur at small scale horizontally and vertically. The large-scale gradients encompass variability in salinity, nutrients, silica, metals, organic matter, oxygen, redox and riverine inputs which occur geographically, topographically, and cross-sectionally. The temporal gradients include short-term (tidally), the medium-term (annually or seasonally) and the long-term (decadally), mainly featuring physical gradients related to turbidity and tidal gradients as well as intertidal/subtidal, resuspension, wave energy, light, particle size, river flow, and the physical effects of the fauna for bioturbation and bio-irrigation (McLusky 1993). The gradients are linked to the transfer of nutrients, oxygen transport, primary production, sediment transport and water filtration, fundamentally determining the diversity of benthic species (Levin et al., 2001; Meire et al., 2005; Palmer et al., 2000). Among the habitats formed in marine soft bottoms, the estuarine and shallow coastal marine habitats are the most geochemically and biologically active areas (Gattuso et al., 2002). They are subject to a variety of stressors associated with direct exploitation and resource use, thus there is an urgent need to ascertain the relationship between diversity of macrobenthos and ecosystem

processes and functions for sustainability of marine soft-bottom habitats (Bolam et al., 2002; Lohrer et al., 2004a; Solan et al., 2004a).

Benthic organisms inhabiting marine bottoms are classified as microbenthos, meiobenthos and macrobenthos according to their size: microbenthos (<32μm) is composed of bacteria and Protista; meiofauna are organisms that are retained on 32μm sieves and are smaller than 1mm encompassing large ciliates as well as metazoa (e.g., rotifers, copepods, oligochaetes, nematodes); benthic macrofauna corresponds to organisms larger than 1 mm in size, contributing to total biomass of sediment community within the density range 10^4 m^{-2} and biomass range 1-100g AFDW m^{-2} (Degraer et al., 2006; Wei et al., 2010). The macrofauna community is dominated by four taxonomic groups: polychaetes, crustaceans, echinoderms, and molluscs (Feller and Nybakken, 2006).

Macrobenthos adopts a wide array of different feeding strategies conditioned by sediment and water properties from which they extract food (Herman et al., 1999; Ysebaert et al., 2003). They occupy different trophic levels including primary consumers, detritivores and predators, with many species able to switch between feeding modes or trophic levels (Herman et al., 1999a). Two major feeding groups are deposit feeders and suspension feeders, whereas grazers, predators and scavenger are less represented in macrofaunal species (Day et al., 2013; Herman et al., 1999a). Deposit feeders rely on the food particles (i.e. particulate organic matter, living cells) on the sediment surface or in the sediment matrix (Kanaya et al., 2008; Riisgård and Kamermans, 2011), and this strategy is used by a majority of the estuarine macrobenthos (Herman et al., 1999a). Suspension feeders filter suspended food particles from the water column, and many polychaetes and bivalves can suspension feed via secretion of mucus or through pumping behaviour (Riisgård and Larsen, 2010). This group is sensitive to changes in water quality

(Weiss et al., 2007), and also has significant impact on the water quality since they filter large amounts of suspended material from the water column (Wall et al., 2008). Grazers mainly feed on benthic microalgae (Hagerthey et al., 2002), and predators feed on other macrofauna or meiofauna (Tita et al., 2000).

Additional groups include interface feeders that switch between suspension and deposit feeding, and omnivores that conduct both predatory and non-predatory behaviour (Herman et al., 1999a; Taghon and Greene, 1992). The switch between suspension and deposit feeding is conditioned by changes in the abiotic environment (i.e. concentrations of suspended particulate matter) or biotic factors (i.e. density) (Taghon and Greene, 1992). Some macrobenthos species exhibit more than two feeding modes, such as euryhaline omnivorous *H. diversicolor* which exhibits carnivory, scavenging, filter-feeding on suspended particles (when sufficient algal cells are present in the water) and deposit-feeding on materials in and on the surface layers of the sediment (Bonsdorff, 2003).

Macrobenthos constitutes a major share of the total sediment biomass in estuarine soft-sediment ecosystems (Day et al., 2013). This benthic invertebrate biomass provides food for higher trophic levels, such as epibenthic crustaceans, fishes and shorebirds, which use the intertidal flats as a nursery area for juvenile stages and/or as adult feeding grounds (McLusky and Elliott, 2004). In a geochemical context, they alter the sediment matrix by particle reworking and burrow ventilation and mediate biogeochemical fluxes (Kristensen et al., 2012). Additionally, macrofauna provides a mechanism for the recovery of organic material by feeding on the bottom materials thus realizing the trophic transfer (e.g. Carlson et al., 1997). Community-related variations of macrobenthos (e.g. species composition, diversity, abundance, and biomass) are correlated well with environmental changes (Veiga et al., 2017), so they are often used to assess the status of

the aquatic environment (e.g. Borja et al., 2000) and to investigate the relationship between biodiversity and ecosystem functioning (e.g. Braeckman et al., 2010; Lohrer et al., 2010).

1.2 Macrobenthos-biogeochemistry

1.2.1 Bioturbation

The functioning of ecosystems (e.g. biogeochemical cycling) is shaped by the interactions between biotic communities and their abiotic environment (Hensen et al., 2006). Bioturbation is the most important interaction between animals and aquatic sediments (Aller and Aller, 1998; Kristensen et al., 2012). Organisms play a global role in the flux of energy and matter across sediment-water interfaces and at global scales virtually all aquatic sediments are affected by bioturbation (Kristensen et al., 2012; Mermillod-Blondin, 2011). In aquatic ecosystems, the most common bioturbators are annelid and priapulid worms, insect larvae, molluscs, echinoderms, benthivore fishes and marine mammals (e.g. sperm whales, narwhals or walruses), and earthworms, insects, mammals and plant roots in terrestrial ecosystems (Meysman et al., 2006a).

Macrofauna is involved in the provision of ecosystem services via mediation of ecosystem functions at the sea-water interface, such as particle mixing and burrow ventilation (i.e. bioturbation) (Kristensen et al., 2012; Rhoads, 1974; Snelgrove et al., 2018). In aquatic ecosystems, bioturbators are characterized by high taxonomic and functional diversity (Holtmann et al., 1996), including a wide array of benthic fauna like crustaceans, polychaete worms, molluscs, echinoderms, brachyopods, sipunculans, cnidarians, priapulida and other meiobenthic faunal organisms (Woodin, 1978, 1974), amongst which the most common bioturbators are annelid and priapulid worms, molluscs, echinoderms and crustaceans (Meysman et al., 2006a; Volkenborn et al., 2012).

Bioturbation is defined as all transport processes carried out by animals that directly or indirectly affect sediment matrices including both particle reworking and burrow ventilation (Kristensen et al., 2012). Particle reworking by macrofauna causes a mixing of the substratum, which is attributed to ingestion and defecation activities, scavenging, as well as burrow construction, maintenance and infilling (Maire et al., 2008). Modes of sediment reworking and mobility of individual species or taxonomic groups are two life traits known to regulate biological sediment mixing (Solan et al., 2014b). Burrow ventilation occurs when organism flush their open- or blind- ended burrows with overlying water for respiratory and feeding purposes, generating a typical non-local bio-irrigation exchange of porewater solutes between the sediment and the overlying water body (Kristensen et al., 2011; Shull et al., 2009) (Figure 1.1).

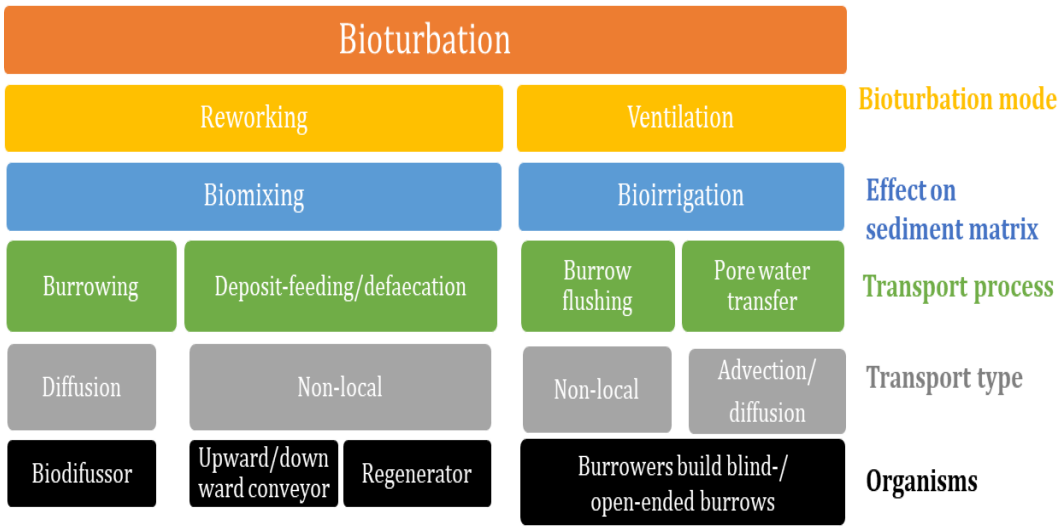


Figure 1.1: Diagrammatic representation of bioturbation as an 'umbrella' term, categories of organisms and overview of bioturbation modes and their effect on sediment matrix, transport process, and transport type. Organisms (in black) can generate different transport types (in grey) so that the grey and black boxes in the figure are horizontally interchangeable. (Modified from Kristensen et al. 2012)

1.2.1.1 Particle mixing

In soft marine sediments, particle reworking activities have profound effects on the physical, geochemical, and biological properties of the substratum (e.g. Krantzberg, 1985; Rhoads, 1974; Ullman and Aller, 1982). Sediment reworking plays a pivotal role in mediating biogeochemical fluxes (e.g. nutrients and O_2) in these habitats by mixing biogenic particles (Gilbert et al., 1996; Kristensen et al., 2012), influencing the burial and delayed release of pollutant distributions (e.g. metals, hydrocarbons) (Gilbert et al., 2001; Reynoldson, 1987; Schink and Guinasso, 1977), and altering the stratigraphic records via relocating sedimentary materials such as microfossils (Aller and Kirk Cochran, 1976; Smith et al., 1986).

The behavioural and morphological traits of macrobenthos determine the capacity of species to disturb the subsurface sediment matrix, and the body size of the organisms also influence their capacity to change the substrate (Queirós et al., 2013; Solan et al., 2004a). Four types of functional groups of sediment reworking have been distinguished (François et al., 1997; Kristensen et al., 2012; Solan and Wigham, 2005): biodiffusors, downward-/upward- conveyors, and regenerators (Gardner et al., 1987) (Figure 1.2 (1)-(5)). Surficial macrobenthos that generates omni-directional particle transport by physiological activities such as feeding and moving are classified as biodiffusors, because the disturbance of the sediment matrix is in analogy to diffusive processes. The non-local transport of upward conveying is conducted by sub-surface deposit feeding benthos, which feed at depth and transport material to the sediment surface (Boudreau, 1997), and the effect is quantified as bioadvection (Gerino et al., 1994). Tubificid oligochaetes (Fisher et al. 1980), the Amphinomida Maldanidae (Aller, 1982) and Arenicolidae (Hylleberg, 1975) are major upward conveyors. Downward conveyors such as Sipunculids are head-upward, vertically oriented species and they cause an “active” non-

local transport from the sediment-water interface to their egestion depth through their gut (Smith et al., 1986). In addition, scavengers such as *Melinna palmata* exhibit conveyor-belt transport of particulates (Queirós et al., 2015). Regeneration is induced by gallery digging species that relocate the sediments from depth to surface such as the fiddler crabs (Gardner et al., 1987). This mode leads to non-local transport of sediments by moving surficial sediments to the bottom of the burrow and a biodiffusive mixing with a high sediment output into the water column by digging (Gerino et al., 2003). Epifaunal species have limited particle reworking capacity in changing sediment characteristics (Figure 1.2 (6)), though they can affect the sediment surface by selective deposit feeding (Alvarez et al. 2013). Given the differences in particle reworking modes among macrobenthos, changes in assemblage structure lead to clear implications for sediment-related processes altered by the fauna.

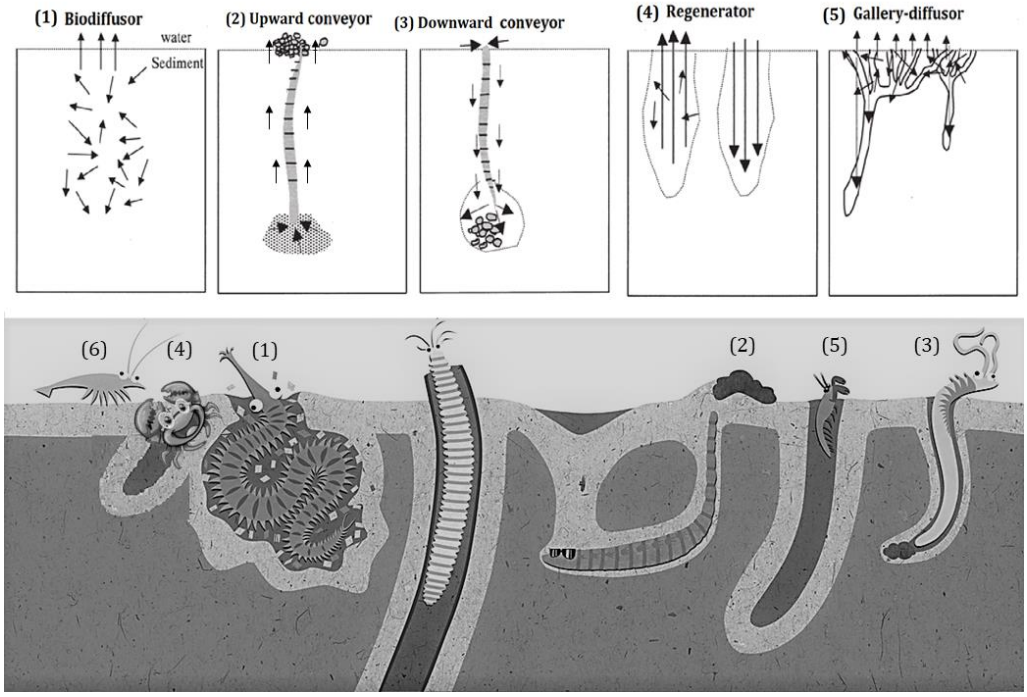


Figure 1.2: Schematic representation of the physical and biological reworking mechanisms of macrobenthos: (1) a goniadid polychaete disturbing sediment in all directions (biodiffusor); (2) a lugworm depositing faecal material at the surface (upward-conveyor); (3) a spionid polychaete depositing faecal material under the surface (downward-conveyor); (4) a crab digging out sediments at depth and moving it

to the surface; (5) a gallery-dweller shrimp (gallery-diffusor); and (6) an epifaunal shrimp with limited bioturbation capacity (Modified from Gerino et al. 2003).

Sediment mixing is not homogenous, because the chemical and physical properties of the sediments can be affected by biota-induced mechanisms (i.e. burrow construction, confined defecation sites and particle sorting mechanisms during feeding) (Kristensen et al., 2012). Intensity of particle reworking by macrobenthos depends not only on biomass, density, and species composition, but also on the relationships between organisms and the abiotic environment (Welsh, 2003). For example, particle reworking was found to be positively influenced by food supply (e.g. algal detritus, faecal pellets) (Graf et al., 1982; Schmidt et al., 2002), particularly intensification of reworking rates has been often linked to spring bloom sedimentation (Boon and Duineveld, 1998; Gerino et al., 2002). Besides, a variety of abiotic factors were demonstrated to influence sediment reworking intensity such as food quality (Dauwe et al., 1998) and temperature (Maire et al., 2006).

1.2.1.2 Ventilation patterns

Rapid exchange of water movement between water and sediment is caused by ventilation of burrow-dwelling organisms such as tube-inhabiting polychaetes, therewith enhancing pore water exchange and solute transfer (i.e. bio-irrigation) (Kristensen et al., 2012). The intensity of burrow ventilation by macrobenthos depends on the purpose (e.g. respiratory need, food filtration, gamete transport, transport of environmental stimuli and removal of metabolic wastes) and the mechanisms vary within and among taxonomic groups (Aller, 1982; Kristensen, 2001). The associated effect of burrow ventilation, bio-irrigation, is determined by factors such as infaunal community composition and sediment type (Aller, 2001; Kristensen, 2001; Meysman et al., 2006b; Shull et al., 2009). More specifically, bio-irrigation is directly affected by body mass and feeding types of the

organisms (Christensen et al., 2000), and also indirectly affected by the morphology (i.e. open-/blind- ended) and composition of the burrow wall (Kristensen et al., 2012) (Figure 1.3).

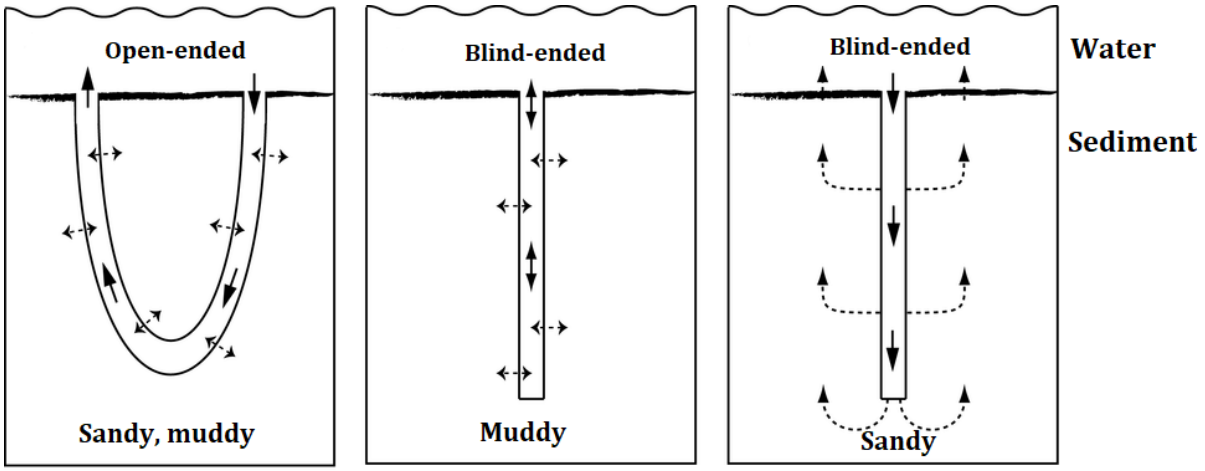


Figure 1.3: The burrow types (open-ended and blind-ended) with ventilation patterns and associated pore water bio-irrigation. (Adapted from Kristensen et al. 2012)

The intermittent nature of bio-irrigation is characteristic for many different types of infauna, such as arenicolid polychaetes, nereid polychaetes (Kristensen, 2001), terebellid polychaetes (Price and Thayer, 1983), thalassinid crustaceans (Volkenborn et al., 2012) and chironomid insect larvae (Polerecky et al., 2006). Species-specific bio-irrigation mechanisms have been observed. For example: bivalves expel water through the exhalant siphon (Volkenborn et al., 2012) and tube dwelling organisms adopted the muscular piston pumping mechanism (Forster and Graf, 1995). Overall, organisms can exert a combination of particle mixing and bio-irrigation dependent on their mobility behaviour and feeding modes.

Determination and quantification of bioturbation (e.g. particle mixing and bio-irrigation) is of great importance in understanding, interpreting and predicting benthic ecosystem functioning (Biles et al., 2010; Suding et al., 2008; Williamson et al., 1999). Necessary research directions were advised by previous studies to achieve this goal: (1) the effect of (changing) community structure on bioturbation and ecosystem functioning due to the current environmental pressure on many habitats and the ongoing species loss at global scale; (2) in-depth studies on most dominant bioturbating species and their influence on ecosystem processes and functions; and on how changing biotic and abiotic factors affect their bioturbation activities (Biles et al., 2010; Ouellette et al., 2004).

1.2.2 Bioturbation effects on sediment biogeochemistry

Ecosystem functioning is defined as the transfer of stocks and fluxes of energy and material between trophic levels and various environments, involving the consumption of the essential nutrients by primary producers and recycling by microbially mediated biogeochemical processes (Godbold, 2012; Hooper et al., 2005). Estuaries support many important ecosystem functions, such as biogeochemical cycling, nutrient movement, purification of water, maintenance of biodiversity and biological production (Costanza et al., 1997; Daily et al.,1997; Meire et al., 1998). Among all the essential processes, the recycling of nutrients through remineralization of organic matter in sediments and subsequent release of part of these nutrients to the water column (i.e. benthic-pelagic coupling) is particularly important for the functioning of shallow coastal and estuarine ecosystems (Brady et al., 2013; Hochard et al., 2012), because they act as strong carbon and nutrient filters, contributing to atmospheric CO_2 and nutrition budget (Borges and Abril, 2012; Laruelle et al., 2010; Regnier et al., 2013). The interaction between macrobenthos and aquatic sediment biogeochemistry is crucial for the global cycles of the carbon, phosphorous nitrogen and other elements (Rhoads and Boyer, 1982;

Snelgrove et al., 2018). The physical and chemical changes in sediments generated by faunal activities include redistribution of particles, water and solutes, causing the spatial and temporal heterogeneity of anoxic, oxic and oscillatory redox zone, and also of the distribution of other electron acceptors (Snelgrove et al., 2018).

Activities of benthic invertebrates strongly influence the biogeochemistry of coastal sediments via bioturbation (i.e. particle reworking and bio-irrigation) (Aller, 2001; Kristensen and Hansen, 1999; Shull et al., 2009a), and composition of macrobenthic community is a key driver in biogeochemical fluxes, due to a variety of different functional traits (Braeckman et al., 2010; Kristensen et al., 2012). Bioturbators physically affect stability of the sediment by enhancing the porosity and permeability of sediments (Sun et al., 2002) via mixing of the sediment particles, bio-irrigation and re-suspension of finer sediment fractions (Montserrat et al., 2009). Bioturbation can alter fluxes of both energy and matter across strong geochemical gradients, in-turn affecting ecosystem functioning such as nutrient cycling, organic matter remineralisation, primary production and sediment transport (Needham et al., 2011). For example, biodiffusors increase nutrient fluxes via destabilizing the substrate and releasing nutrients into the porewater when they disturb sediment matrix in all directions, and upward-/downward-conveyors regulate the burial and release of organic matter when they transport materials out or into the sediment matrix (Kristensen et al., 2012). Bio-irrigators promote sediment aeration and stimulate aerobic microbial activity whilst introducing oxygen rich water to greater depths (Botto and Iribarne, 2000; Kristensen, 1985). The introduction of oxygen rich water leads to redox heterogeneity, tightening coupled nitrification-denitrification and having major implications for the cycling of sulfur, and organic carbon compounds in marine systems (Mermillod-Blondin, 2011; Snelgrove et al., 1998). Furthermore, bioturbators can transfer excretion products and metabolites

deeper into the sediment and dislocate organic contaminants and heavy metals (Gilbert et al., 1994; Selck et al., 2005). In general, these processes stimulate mineralization and increase nutrient turnover accordingly, which enhances primary production due to the renewal of the nutrient pool (Blackburn, 1988). Changes in community structure, density-dependent processes, and interactions between species could contribute significantly to geochemical variability in sediments at the community level (Karlson et al., 2007; Michaud et al., 2005; Nizzoli et al., 2007; Nordström et al., 2006; Waldbusser et al., 2004). To understand ecosystem functioning of estuaries, it is therefore essential not only to understand the underpinning species' mechanisms of bioturbation at individual level, but also to monitor biodiversity and the biotic interactions at community level.

In addition, environmental parameters such as temperature, chemical composition of the sediment (e.g. organic matter content, C:N:S ratio, iron content) and hydrological characteristics of the sediment system largely condition the effects of bioturbation on sediment biogeochemistry (Queirós et al., 2013). It has been reported that sediment biogeochemistry, particularly oxygen consumption of the sediment community, can be predicted by grain sizes and levels of hydraulic conductivities between overlying water and pore water (Mermillod-Blondin and Rosenberg, 2006). In systems governed by diffusive pore water flows attributed to low hydrological conductivity, benthic bioturbators might act as direct vectors and flux modulators of solute fluxes; whilst in sediments with high hydrological conductivity, the promoting effect of macrobenthic bioturbation on biogeochemistry can be overruled (Mermillod-Blondin, 2011). Thus, in gaining insight in bioturbation effects on sediment biogeochemistry, it is of utmost importance to understand the interplay between biotic components and abiotic factors (e.g. physical and geochemical parameters) and the changes across environmental gradients.

1.3 The Scheldt estuary

Cited among the most productive environments, coastal marine and estuarine ecosystems are also important life-support systems for human beings (Costanza et al., 1997; Day et al., 2013). Geomorphologically, estuaries are highly dynamic and open systems connecting rivers and sea, thus forming a variety of different habitat types. There are physical, chemical and biological links between the habitats formed in the estuarine systems. The major habitat types are subtidal sediments, intertidal mudflats, intertidal sandflats, lagoons, sand-dunes, marshes and coastal grassland (Barnes, 1974). Many estuaries experience varying environmental and biotic conditions which are mediated by water movement over short time scales, such as temperature, salinity, chemicals and plant and animal densities (Day et al., 2013; McLusky and Elliott, 2004). Additionally, present-day estuarine systems are facing increased human population densities and consequent exploitation and alteration of the environment (e.g. damming, dredging, dumping, and land reclamation), which conflict strongly with the high biological values (Jeuken and Wang, 2010; Lesourd et al., 2007; Winterwerp et al., 2002).

The study site of this thesis, the Scheldt estuary, is a typical example of such a system. Situated in NW-Europe across the national border of Belgium and the Netherlands (Figure 1.4), the Scheldt estuary extends from its mouth in the North Sea in Vlissingen to Ghent, where tidal waves are impaired by sluices (Meire et al., 2005). The river Scheldt has a length of 355 km from the south (France, St. Quentin) to mouth, and the total catchment area is approximately 21.863 km^2. The Western Scheldt (58 km) is the middle and lower part of the estuary (the Dutch part), and it is a well-mixed region characterized by complex morphology (Meire et al., 2005). The length of the tidal river amounts to 235 km, entering major tributaries of the Rupel and Durme rivers in the Belgian part of the

estuary that is characterized by a single ebb/flood channel bordered by mudflats and marshes (the Zeeschelde) (Meire et al., 2005).

Since the river Scheldt is a rain-fed river, the river discharge varies seasonally, ranging from 180 m^3 s^{-1} in winter to 60 m^3 s^{-1} in summer (Baeyens et al., 1997). The residence time of the water is one to three months, depending on the river discharge (Soetaert and Herman, 1995). With a funnel-shaped morphology, the mean vertical range of tides is maximal in the freshwater tidal reaches (5.24 m) (Claessens, 1988). During an average tidal cycle, the maximum tidal velocity is 0.9 m s^{-1} at the mouth, 1.1 m s^{-1} in the Zeeschelde and 1.2-1.3 m s^{-1} between Antwerp and the Rupel.

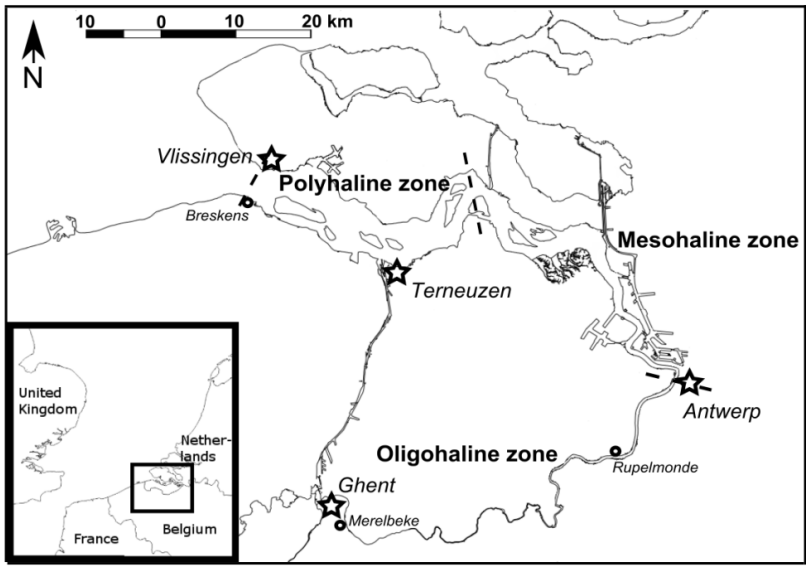


Figure 1.4: Map of the Scheldt estuary and its tributaries. The three salinity zones of the estuary (polyhaline, mesohaline, and oligohaline) are separated by dash-dot lines

The vertical salinity gradients of the Scheldt estuary are almost negligible since the estuary is well-mixed. In contrast the longitudinal salinity profile of the estuary varies between fresh and salt water due to the influence of dynamical river discharge (Damme et al., 2005; Soetaert et al., 2006). Three salinity zones are delineated: the polyhaline zone

(average salinity >18) of Scheldt stretches out from the mouth (0 km) to Hansweert (40 km); a mesohaline zone (average salinity between 5–18) is situated between Hansweert to Dutch-Belgium border (58 km) with a steep salinity gradient; the oligohaline zone reaches the upstream of Rupel with the salinity range 0-5 (Meire et al., 2005; Ysebaert et al., 2003).

During the last centuries, major geomorphological changes occurred to the Scheldt estuary, attributed to the reclaiming of tidal marshes for building agricultural land in middle ages and more recently the industrial and urban developments since the middle of the 20th century. About 16% of surface area was reported as lost in last century due to the embankment of the marshes and dike building (Meire et al., 2005). Intensified, large-scale dredging took place to guarantee the safe access to the port of Antwerp along the Scheldt estuary, and most dredged material is relocated within the estuary at some specified dumping locations. This has already led to the decrease of mud flats (low dynamic area) and increase of sand flats (high dynamic area), and the morphological evolution of the habitats such as the substantial increase of tidal amplitude near Antwerp is foreseen for the future (Meire et al., 2005). To avoid further tidal amplification, particularly to investigate how navigability can be improved without negative effects to nature and safety against flooding in the Scheldt estuary, the SIGMA-plan has been carried out under the framework of the project “Integrated Plan Upper-Seascheldt” (van Holland et al. 2015). Mitigation of the negative impacts of historical anthropogenic changes and positive evolution of the Scheldt estuary have been confirmed from the observed changes in water level, bathymetry, habitats (salt marshes) and sediment concentrations since 2005 (van Holland et al. 2015).

Amongst the most nutrient-rich systems in the world, the Scheldt estuary undertook serious degradation of surface waters (i.e. oxygen depletion and intense water-column

denitrification in the upstream part and intense nitrification downstream) in the second half of the 20th century due to high loadings of ammonium and organic matter and ineffective local water management (Soetaert et al., 2006). Particularly in early 1970s, symptoms such as temporal anoxia and organism mortality were common features in the upstream part of the Scheldt. Efforts have been made in the late 1970s, including the implementation and upgrading of water treatment and the imposition of restrictions on the disposal of wastes by industries, leading to a gradual improvement of the water quality, suspended matter and sediments, and the reappearance of organisms in the Scheldt estuary (Appeltans, 2003; Baeyens et al., 1997; Soetaert et al., 2006). Soetaert et al. (2006) reported conservative behaviour of nitrate (i.e. the sinks almost balanced the sources) over the entire estuary until the beginning of the 21st century.

The abovementioned abiotic characteristics (e.g. hydrodynamics, geomorphology and biogeochemistry) shaped the biotic communities (i.e. benthic invertebrates, fish, water birds) in the Scheldt estuary. Feeding groups of macrofauna vary along the salinity gradients, characterized by the dominance of biomass by suspension feeders in the marine part and deposit feeders in brackish part (Ysebaert et al., 2003). Due to the improved water quality and restoration of migrating species, increasing number of water birds, fish, mysid shrimps and higher benthic biomass have been reported (Maes et al., 1998; Meire et al., 2005; Rappé et al. 2011).

In the Western Scheldt, distinct spatial and temporal variation of macrobenthic communities was indicated by previous studies (Ysebaert et al., 2003). In the polyhaline zone, diversity is higher and seasonal variation is less pronounced compared with mesohaline zone, whilst the benthic community in the oligohaline zone is restricted to oligochaetes (Seys et al., 1999).

1.4 Using macrobenthos for ecology conservation and estuarine ecosystem management

Estuaries have the highest degree of urbanisation, and the recognition of its unique functional and structural biodiversity values adds weight on the anthropogenic impact on estuaries (Meire et al., 2005). It is particularly important for integrating ecological management with sustainable development of economy, with a focus on ecosystem health. Ecosystem services are defined as the contributions that ecosystems make to human well-being which retain a connection to the underlying ecosystem functions, processes and structures that generate them (Haines-Young and Potschin, 2012). Macrobenthos mediate important ecosystem functions such as nutrient cycling and primary and secondary productivity (Herman and Heip, 1999; Ingels et al., 2014; Levin et al., 2001), which underpin regulation and maintenance of ecosystem services (Haines-Young and Potschin, 2012).

Ecosystem health encompasses its ability to provide ecosystem services, sustainability of economic activity while maintaining ecological integrity and the structure and function maintained in response to stress (Rapport et al., 1998). The increasing pressure of human-driven large-scale disturbances on coastal seascapes, such as climate change and overfishing, have generated concern among researchers and marine managers about the functional consequences of benthic biodiversity loss (Lohrer et al., 2004a). These disturbances can have drastic effects on ecosystems; for example, bottom trawling and dredging would eliminate habitat-forming benthic biota and remove big macrobenthic bioturbators from large areas in the seafloor, resulting in habitat losses and noticeable reductions in environmental heterogeneity (Thrush and Dayton, 2002). Loss of key bioturbating species will possibly cause the decline in favourable niches for other infauna

due to changes in oxygenation and metabolite removal (Austen et al., 1998; Austen and Widdicombe, 1998; Widdicombe et al., 2004). Macrobenthos modulates ecosystem functions related to sediment processes by generating bioturbation activities (Kristensen et al., 2012; Herman et al., 1999b). For example, bioturbators can counteract nitrogen eutrophication by stimulating higher coupled nitrification-denitrification rates along burrow walls and releasing dinitrogen gas through nitrogen cycle in the sediment (Stief, 2013; Seitzinger, 1988). Besides, macrobenthos are considered to be good indicators of the status of the estuarine environment, because they exhibit different tolerances to environmental gradients and most of them cannot migrate out of their habitat (Dauer, 1993) being also sensitive to pollution (Eggleton and Thomas, 2004). Therefore, the disturbance of bioturbators can have far-reaching impacts at the ecosystem level (Solan et al., 2004a).

Significant efforts are devoted to understanding consequences from anthropogenic stressors on essential ecosystem functions (Diaz and Rosenberg, 2008; Solan et al., 2004a). It has become increasingly urgent and important to develop assessment approaches for ecosystem-based management as global anthropogenic pressures on natural environments continue to grow and management focus shifts to address issues of loss of function and concomitant ecosystem services. Ecosystem-based monitoring approaches should include the full array of interactions that occur within an ecosystem, including not only species, or ecosystem services in isolation, but also human impact (Levin et al., 2009; Levin and Lubchenco, 2008). Due to the cascading effects of some macrobenthos on the wider system, the functionally important species should be the conservation targets (Crain and Bertness, 2006), thus identifying and preserving the key species and responsive ecosystems is a key priority for conservation (Byers et al., 2006). According to Braeckman et al. (2014), incorporating the group of common (i.e. key)

macrobenthic species would contribute considerably to the concept of ecosystem-based management in the marine realm.

1.5 PhD objectives

1.5.1 Research gaps

While much work has been done on improving a thorough understanding of estuarine ecosystem processes and functions, a good and sustainable management also depends on the assessment of ecosystem functioning at a broad scale. Scaling up the effects of local processes to a larger context is essential to ecology (Kerkhoff and Enquist, 2007; Levin, 1992; Peterson et al., 1998), however, there is a considerable gap in our knowledge of bioturbation effects on ecosystem functioning at the landscape scale. Particular challenges are that : (1) the dominant drivers of functioning may shift across scales in heterogeneous environments (Hewitt et al., 2007; Sandman et al., 2013; Snelgrove et al., 2014); and that (2) the environmental heterogeneity increases with the scale, leading to inaccuracy to incorporate heterogeneity in extrapolations (Godbold et al., 2011; Needham et al., 2011; Peterson, 2000). Thus, integrating detailed observational and experimental studies conducted in a range of environmental contexts is essential to evaluate space-, time-, and habitat-dependent influences of bioturbation on ecosystem functioning in estuarine and shallow coastal zone.

From an ecological point of view, the Scheldt estuary is a unique and exceptional system in Europe. It not only presents a full gradient from fresh water to salt water with high dynamics but also includes a 60km freshwater tidal area with diverse fauna and flora; from the perspective of ecosystem management, the intense anthropogenic alteration has led to severe environmental issues such as pollution, eutrophication and morphological changes (Meire et al., 2005).

Classification of ecosystems or geographic areas into distinct spatial units on the basis of geographical and ecological criteria is useful for policy and conservation purposes in environmental studies (Dankers et al., 2012; Frissell et al., 1986). Ecotopes are more recognizable, relatively homogeneous natural units which represent potential species niches and the general status of abiotic and anthropogenic local conditions (Hawkins et al., 2010). The ecotope map of the Scheldt estuary (Figure 1.5a; Twisk, 2002; Leuven et al., 2018) has been well-established by following the ecotope system for coastal waters ZES.1 typology (Bouma et al., 2005; Kers et al., 2013). This ecotope classification system hierarchically arranges the ecotope variables on the basis of the dominance of the physical environmental factors and processes (mean and variability of salinity, substratum type, mean water depth, and hydrodynamics) in determining community composition. Sampling sites in this thesis were chosen according to salinity and habitat (Figure 1.5b). Within each salinity zone (polyhaline - P, mesohaline - M, oligohaline - O), the locations were selected based on their proximity to each other (ensuring similar water column properties, i.e. salinity, temperature and oxygen concentration) (Cleveringa, 2014; Van Ryckegem et al., 2014). Only high-dynamic subtidal habitats were sampled due to the safety consideration that low-dynamic habitats were located too close to the shore to sample with a research vessel. Referring to Ecotope maps is of great help because they provide a more integrative understanding of the study site, thus the sampling framework can be established to target the spatio-temporal variability in macrobenthic communities (Ysebaert et al., 2003). Therefore, environmental properties that could affect distribution and behaviour of macrobenthos such as temperature, salinity, sediment types, food supply and food quality (Ouellette et al., 2004; Cruz-Rivera and Hay, 2000; O'Connor et al., 2015) were considered in choosing sampling sites in the baseline survey of this thesis (Figure 1.5b; **Chapter 2-3**). Sampling sites in this thesis were chosen according to salinity and habitat (Figure 1.5b). Within each salinity zone

(polyhaline - P, mesohaline - M, oligohaline - O), the locations were selected based on their proximity to each other (ensuring similar water column properties, i.e. salinity, temperature and oxygen concentration) (Cleveringa, 2014; Van Ryckegem et al., 2014). Only high-dynamic subtidal habitats were sampled due to the safety consideration that low-dynamic habitats were located too close to the shore to sample with a research vessel. Referring to Ecotope maps is of great help because they provide a more integrative understanding of the study site, thus the sampling framework can be established to target the spatio-temporal variability in macrobenthic communities (Ysebaert et al., 2003). Therefore, environmental properties that could affect distribution and behaviour of macrobenthos such as temperature, salinity, sediment types, food supply and food quality (Ouellette et al., 2004; Cruz-Rivera and Hay, 2000; O'Connor et al., 2015) were considered in choosing sampling sites in the baseline survey of this thesis (Figure 1.5b; **Chapter 2-3**).

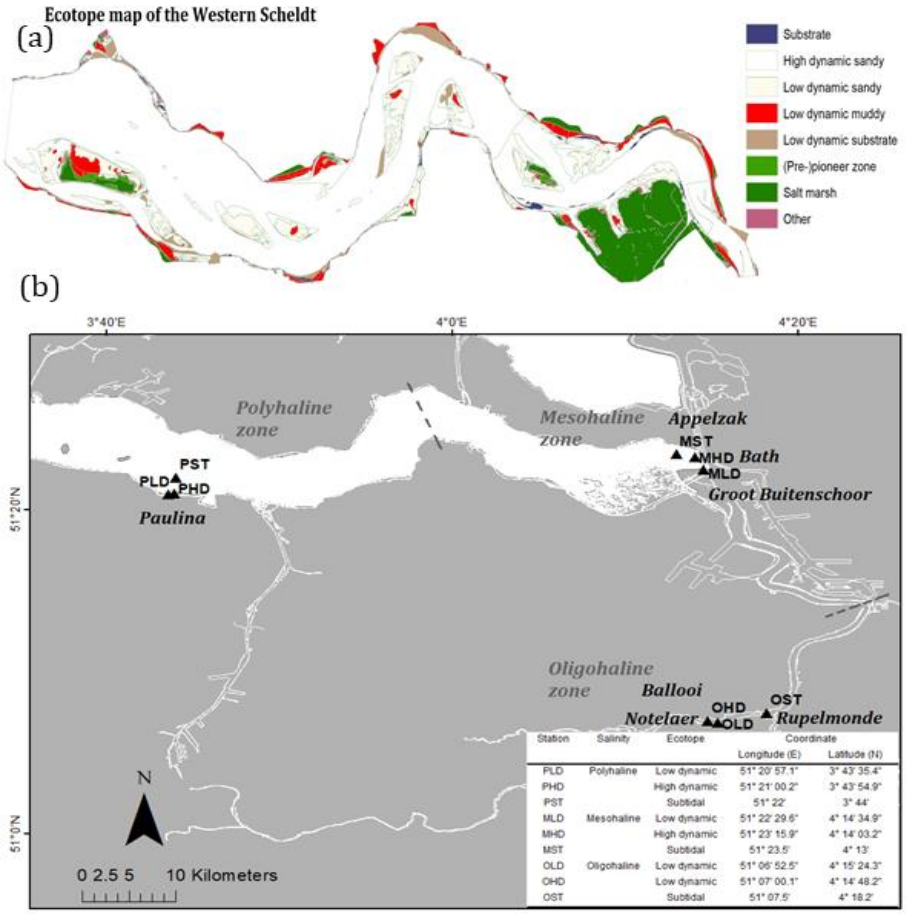


Figure 1.5: Ecotope-driven choice for the sampling sites in the Scheldt estuary in this thesis (a) Ecotope of the Scheldt estuary (2012) (modified from Leuven et al. 2018) (b) Sampling sites in the baseline survey of this thesis, which were decided based on the ecotope map.

In an ever-changing system like the Scheldt estuary, human impact has been putting more weights on deciding the future status of the ecosystem. In the past century, the Scheldt was confronted with negative consequences (e.g. eutrophication, species loss) induced by human activities, whilst efforts made by human beings led to positive changes (e.g. water treatment, habitat restoration). In the future, inevitable challenges remain including the necessity of deepening the channel and infrastructure of flood protection due to the consideration of economy and safety in the Scheldt (Meire et al., 2005). Therefore, it is more than ever urgent to develop management plans for sustainable

development of the estuary. To achieve this goal, the pre-condition is to gain insight and predict the response of the estuarine ecosystem functioning to human-induced hydro-morphological and biogeochemical changes. Macrobenthic bioturbators offer an ideal model system to investigate benthos's effect on ecosystem functioning, since they have disproportionate effects on ecosystem functioning and services (Jones et al., 1997, 1994; Wright and Jones, 2006). Especially in the Scheldt estuary, the spatio-temporal distribution patterns and diversity of macrobenthos have been thoroughly studied (e.g. Meysman et al., 2003; Van Der Wal et al., 2008; Ysebaert et al., 2005, 1993; Ysebaert and Herman, 2002; Ysebaert and Meire, 1998).

1.5.2 Outline

This PhD thesis aims to provide a comprehensive spatio-temporal assessment of the roles of macrofauna in estuarine biogeochemistry in the context of natural heterogeneity and anthropogenic disturbance in the coastal marine and estuarine environments. The thesis adopts a multidisciplinary field, laboratory - based and model integration approach (Figure 1.6).

This thesis is composed of a general introduction (**Chapter 1**), four topical chapters (**Chapter 2-5**) and a general discussion (**Chapter 6**). **Chapter 2-5** are research articles and can be read separately. **Chapter 2** and **3** have a shared first authorship with Sebastiaan Mestdagh (NIOZ), and in **Chapter 4** and **Chapter 5** the PhD candidate is the sole first author.

The general introduction (**Chapter 1**) sets the background of environment-benthos-ecosystem functioning relationships and the promising assessment of ecosystem management from a benthic perspective. The baseline knowledge of bioturbation effect on biogeochemistry is built in **Chapter 2** and **Chapter 3**, and the link between the *in situ* macrobenthic community, their bioturbation activities, and the resulting biogeochemical

fluxes were established from the direct rate measurements of sediment-water exchange of nutrients and bioturbation in mesocosms for the different communities in the Scheldt estuary, in different seasons of the year. **Chapter 2** describes how variation in key species drive bioturbation (i.e. particle reworking and burrow ventilation) along the estuarine gradients in different seasons; whilst in **Chapter 3** focus is placed on the contributions of environmental and macrofaunal properties to the spatio-temporal variability in biogeochemistry. Based on the insights gained from **Chapter 2** and **Chapter 3**, **Chapter 4** explores the importance of biological traits and population density of two key bioturbators (*H. diversicolor* and *L. balthica*) to oxygen dynamics in two contrasting sediments. In **Chapter 5**, I joined the points addressed from **Chapter 2**-**4** to produce the spatial mapping of benthos-mediated oxygen dynamics at the estuarine landscape scale with direct applications for ecosystem management, by integrating biomass scaling of faunal-mediated O_2 uptake and species distribution modelling. In the final Chapter (**Chapter 6**), a synthetic discussion about benthos contribution to ecosystem functioning across space and time was constituted based on the key findings from **Chapter 2-4** and the application of the integrated modelling approach applied in **Chapter 5** in accessing estuarine ecosystem management was evaluated. Reflections of this study and outlook for future researches are presented.

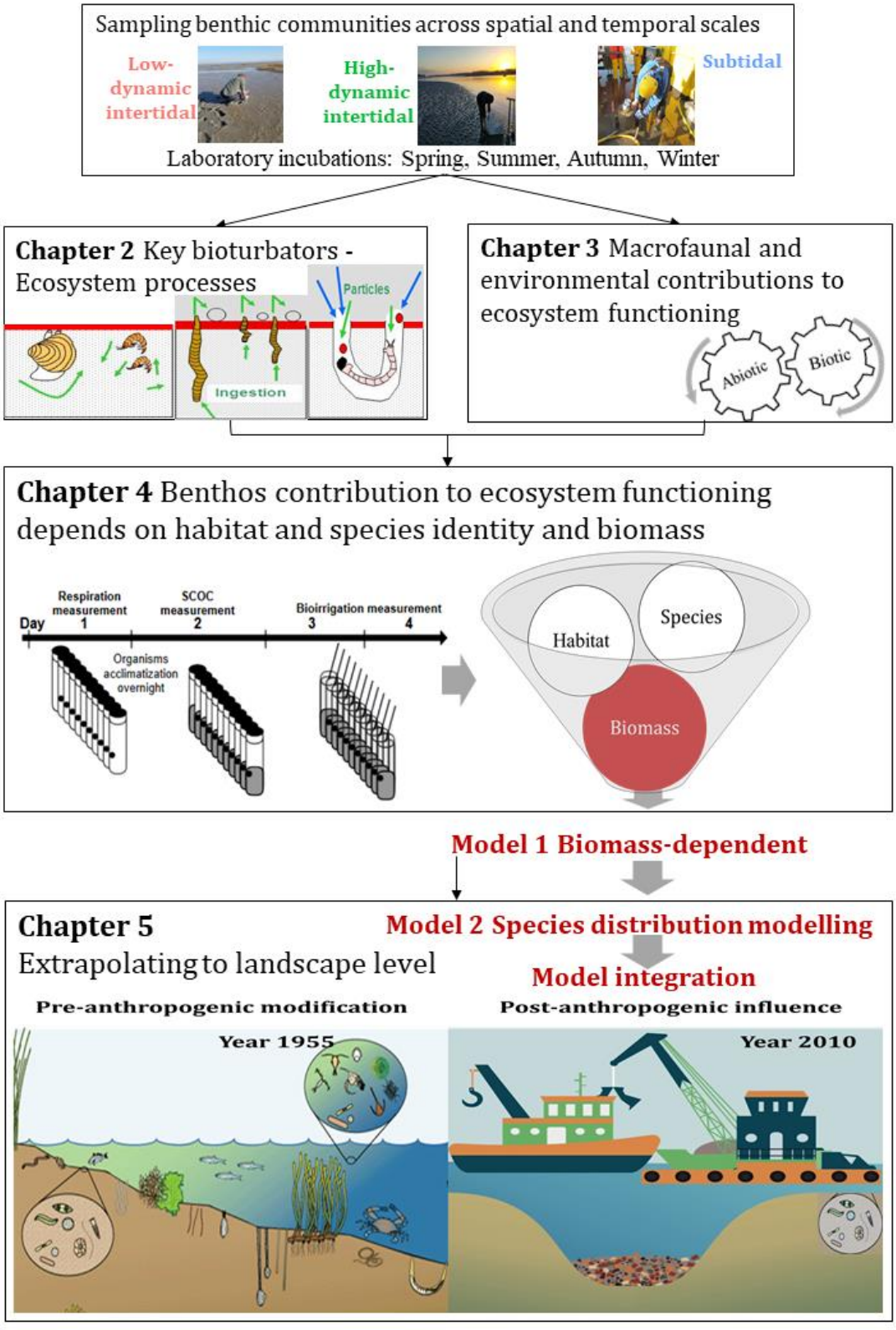


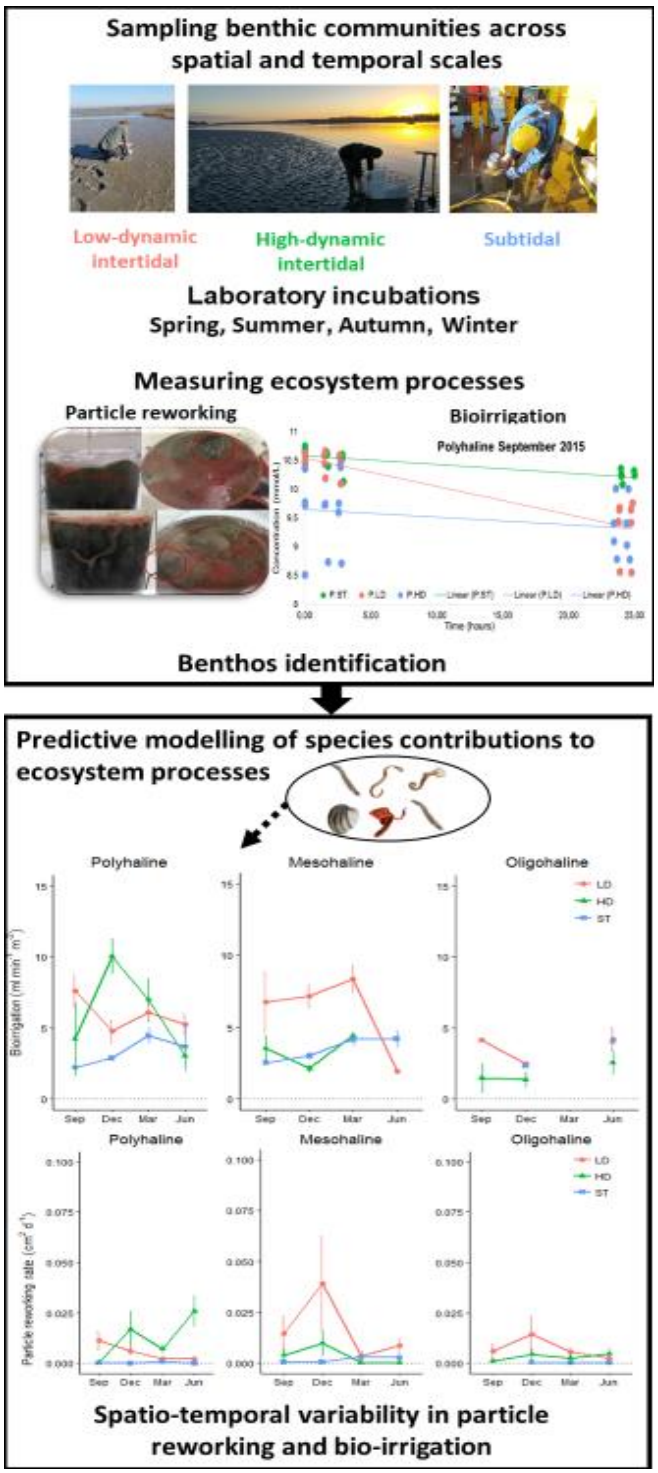
Figure 1.6: A schematic diagram presenting the overview of the structure and the holistic research approaches of the thesis

Chapter 2 Spatio-temporal variation in sediment ecosystem processes and roles of key biota in the Scheldt estuary

Abstract

Bioturbation (i.e. particle reworking and bio-irrigation) is a key process in benthic coastal and estuarine ecosystems, and acts as a crucial control on important ecosystem functions, such as nutrient cycling. Particle reworking and bio-irrigation are affected by a wide array of environmental and biological factors and are therefore expected to be highly variable in heterogeneous ecosystems, such as estuaries. To assess the spatial and temporal variability of these benthic ecosystem processes in the Scheldt estuary (Belgium and the Netherlands), we carried out seasonal surveys during four seasons in 2015 and 2016, at three habitats with different hydrodynamic regimes (low-dynamic intertidal, high-dynamic intertidal, subtidal) in the three main salinity zones (polyhaline, mesohaline, oligohaline). Sediment communities were sampled and incubated in the laboratory; macrobenthos was identified and weighed. Luminophore tracers were applied to incubation cores to measure sediment particle mixing and changes over time in the concentration of the inert tracer sodium bromide in the water column were used to estimate pore water exchange rate as a proxy for bio-irrigation. Particle reworking was quantified both by the rate of mixing, and the maximal penetration depth of luminophores. Luminophore profiles indicated that biodiffusion was the dominant particle reworking mode. Both particle reworking and bio-irrigation were highly variable among seasons and habitats, and different species were found to contribute unevenly to both processes, and between habitats and seasons. Habitat structuring effects on populations, density-dependent interactions with the habitat, and temperature-driven

variability in macrobenthos activity and living position are suggested to explain the observed spatial and temporal differences.



Graphical abstract of Chapter 2

This chapter is adapted from the publication:

Fang, X., Mestdagh, S., Ysebaert, T., Moens, T., Soetaert, K., Van Colen, C., 2019. Spatio-temporal variation in sediment ecosystem processes and roles of key biota in the Scheldt estuary. Estuar. Coast. Shelf Sci. 222, 21–31. https://doi.org/10.1016/j.ecss.2019.04.001

2.1 Introduction

Ecosystem nutrient cycling determines productivity and water quality in shallow coastal habitats, and depends on biotic and abiotic processes that redistribute particles and modify fluxes of solutes at the sediment-water interface (Braeckman et al. 2014; Godbold 2012; Hiddink et al. 2009; Queirós et al. 2011). The importance of macrofaunal activities for these processes has been recognised since Darwin's work on earthworms and soil formation (Darwin, 1881). Over the last two decades, benthic ecologists have increasingly provided evidence about how these benthic animals affect sediment biogeochemistry, e.g. via facilitation of nutrient cycling (Aller, 1982; Blackburn, 1988; Raffaelli et al., 2003; Rhoads, 1974; Snelgrove et al., 2014; Solan et al., 2004a).

Modification of sediments through macrofauna-mediated transport can be separated into two main processes: particle reworking (Maire et al., 2008; Solan and Wigham, 2005) and bio-irrigation (Kristensen and Kostka 2005; Meysman et al. 2006b). In addition to mixing particles and associated living and non-living substances through faunal feeding, defaecation and burrowing activities (i.e. particle reworking), sediment-dwelling infauna also ventilate their burrows with seawater and dissolved substances for respiration and feeding purposes (i.e. bio-irrigation; (Kristensen et al., 2012; Riisgård and Larsen, 2005). Particle reworking and bio-irrigation are grouped under an umbrella term bioturbation, which is defined as all "transport processes carried out by animals (and plants) that directly or indirectly affect sediment matrices" (Kristensen et al. 2012). However, both processes can be interrelated when species modify the sediment matrix by mixing particle and ventilating their burrows simultaneously (Vopel et al. 2007). For example,

fine-grained particles can be transported into sediments by bio-irrigating organisms (e.g. Rusch and Huettel 2000; Wethey and Woodin 2005). Also, the ecosystem engineering effects of macrofauna such as lugworms via reworking and burrow ventilation have long been recognised (Riisgård and Banta, 1998). Since the effect of bioturbation on biodiversity and ecosystem functioning is a combination of many complex processes, it is useful to evaluate the different effects resulting from the activities of benthic organisms separately (i.e. bio-irrigation or particle reworking) (Gerino et al., 2002; Kristensen, 2000; Martin and Banta, 1992). Nevertheless, both processes have rarely been investigated simultaneously in natural communities (but see Hedman et al., 2011; Mestdagh et al., 2018a). Consequently, it is poorly understood how both processes vary over spatial and temporal environmental gradients.

In marine and estuarine soft sediments, the macrobenthos, which covers a range of biological traits that influence particle reworking and bio-irrigation, differentially affects chemical transport regimes of dissolved and particulate matter (Vopel et al., 2003), making them bioturbators and bio-irrigators (Boudreau, 1998).

In addition to their direct participation to sediment diagenesis via aerobic respiration and metabolite excretion (Vanni, 2002), the macrobenthos also provides microbial habitats for intensified geochemical and redox oscillation and gradients (Sundbäck et al., 2004), that stimulate sediment nutrient cycling and metabolism (Van Colen et al., 2012). The contributions of species to sediment mixing and irrigation are variable, and depend on population demography (Gerino et al., 2003), i.e. size, density of life stages (Matisoff et al., 1982; Sandnes et al., 2000) and biomass (Nizzoli et al. 2002; Solan et al. 2004a), and on species-specific traits such as feeding behaviour and mode of movement (Aller, 2001).

In general, three modes of sediment particle mixing have been observed in research performed with monospecific populations: biodiffusion (Goldberg and Koide, 1962), bio-

advection (Fisher et al. 1980; Rice 1986; Robbins et al. 1979) and regeneration (Benninger et al., 1979; Gardner et al., 1987; Sharma et al., 1987). Omnidirectional particle transport over short distances by macrobenthos is referred to as biodiffusion (Cochran, 1985; Guinasso and Schink, 1975; Wheatcroft et al., 2008). Bio-advection is the non-local transport produced by conveyor belt feeders (Rhoads, 1974), such as some polychaetes and oligochaetes that feed at depth and egest at the sediment surface (Boudreau, 1997). The burial rate and advection of surface sediment by intensive faeces deposition usually outweigh physical sedimentation rates (Gerino et al. 1994; Robbins et al. 1979). Regeneration results from the subsequent filling of deserted burrows with fresh surface sediment, or from the subsurface egestion by inverse conveyor belt feeding at the sediment surface (François et al., 1997; Smith et al., 1986).

Modes of burrow ventilation and associated pore water irrigation by macrofauna vary greatly between individuals due to intra- and interspecific differences in biological traits, such as body size, feeding mode, living position or burrow type (Renz et al., 2018; Wrede et al., 2018), and physiological differences in respiration and removal of metabolic products (Kristensen, 2001). For example, deep (> 15 cm) and intense burrow ventilation by *Arenicola marina* stimulates the aerobic microbial metabolism more than the shallower irrigation (< 10 cm) by *H. diversicolor* in sandy sediments (Banta et al. 1999). Similarly, differences in burrow structure and ventilation behaviour between *Marenzelleria viridis* and *H. diversicolor* have contrasting effects on carbon and nitrogen cycling in coastal sandy sediments (Vasquez-Cardenas et al., 2016). The strong ventilation in the open-ended burrows of *H. diversicolor* creates more oxidised conditions in deeper sediments as compared to the weaker ventilation by *M. viridis* in blind-ended burrows (Kristensen et al., 2011). However, burrow ventilation by *M. viridis* induces advective pore water transport, stimulating sulphate reduction in deeper sediments and

enhancing sulphide oxidation in surface sediments (Kristensen et al., 2011; Quintana et al., 2011).

Estuaries are highly heterogeneous and dynamic systems with strong spatial and temporal environmental gradients (McLusky et al., 1993). Spatial variation in the macrofauna communities is typically associated with large-scale patterns in salinity, oxygen and the hydrodynamic regime that determines sediment composition of the different benthic habitats (Fraschetti et al., 2002; Ysebaert and Herman, 2002). Temporal patterns in community properties, on the other hand, are associated with the seasonal dynamics of juvenile recruitment (Connell, 1985), mortality (Van Colen et al. 2010a), and dispersal (Boesch and Rabalais, 1991). Moreover, estuaries possess strong seasonal variation in salinity, oxygen, sediment composition and organic matter loading at the local habitat scale, to which benthos activities are highly sensitive (Clark et al., 2011; Godbold et al., 2011; Godbold and Solan, 2013; Langenheder et al., 2012). For instance, food availability (Törnroos et al., 2015) and temperature (Ouellette et al., 2004) are known to affect benthos-mediated fluxes of solutes. Other studies found that particle reworking can have different effects on biogeochemistry depending on sediment type (Mermillod-Blondin and Rosenberg 2006; Volkenborn et al. 2010).

In this study, particle reworking and bio-irrigation were quantified in benthic communities sampled seasonally in different habitats and salinity zones in the Scheldt estuary (Belgium and the Netherlands). The objective of this study was to determine the spatio-temporal patterns in particle reworking and bio-irrigation and to quantify the relative contributions of the macrofauna species to these processes. We hypothesized that (1) the biomasses of the dominant macrobenthos can significantly predict the spatio-temporal pattern of the examined ecosystem processes (sediment particle reworking and

porewater exchange), and that (2) species can contribute differently to both processes depending on their functional traits and the spatio-temporal environmental context.

2.2 Materials and Methods

2.2.1 Study sites, sampling and experimental set-up

Sampling sites were chosen along the Scheldt estuary (Belgium and the Netherlands) according to salinity and habitat (Figure 2.1). Three major salinity regimes were identified: polyhaline – P (mean salinity > 18); mesohaline – M (mean salinity between 5 - 18); and, oligohaline – O (salinity < 5) and based on previous classification by Meire et al. (2005) and Ysebaert et al. (2003). Within each salinity regime, the benthic communities of three different habitats were sampled, where habitats were classified based on modelled current velocities (subtidal - ST, high-dynamic intertidal - HD, low-dynamic intertidal – LD), although grain size and mud content differs among these habitats within salinity regimes (Twisk, 2002; van der Wal et al., 2017; also refer to survey data presented in this PhD thesis **Chapter 3** Table 3.1). Four replicate Plexiglass cores were collected with a sampling area 2 m^2 in each habitat type and salinity regime (N = 36) (Supplementary material Figure 2.1). This framework was assigned to target the spatio-temporal variability in macrobenthic communities (Ysebaert et al., 2003) and environmental properties that could affect benthos behaviour such as temperature (Ouellette et al., 2004), salinity, food supply and food quality (Cruz-Rivera and Hay, 2000; O'Connor et al., 2015). Sediment communities were sampled to a depth of 10 cm at 8 sites in September and 9 sites in December 2015 and March and June 2016 along the salinity gradient in the Scheldt estuary (Figure 2.1). In September 2015, sediment cores from oligohaline subtidal habitats were not successfully collected due to the difficult access with the research vessel.

In order to create a sufficient pressure gradient to ensure advective water flows through the sediment (Glud et al., 1996; Huettel and Gust, 1992), two types of corers were used in sample collection: small cores with magnetic stirring rods (Ø 9 cm, height 25 cm) or large corers with stirring discs (Ø 19 cm, height 30 cm). The core type was selected based on the perceived permeability (the observed grain size) when the sediment was sampled during the first sampling campaign in September. Eventually, the communities inhabiting the permeable sediments in the high-dynamic habitats in the poly– and oligohaline zone were sampled with a large corer (19 cm inner diameter), while the other finer grained sediments were sampled with a smaller corer (9 cm inner diameter).

Subtidal communities were collected on board the RV Simon Stevin (VLIZ – Flanders Marine Institute) using a NIOZ box corer (Reineck Box corer with Box dimensions: 500 x 500 mm, L = 550 mm; Empty weight: 1350 kg), and intertidal communities were collected with Plexiglas corers during low tide when the flats were accessible on foot. In situ seawater was collected via 60L barrels from each salinity zones (3 barrels per salinity zone) during high tide.

The mesocosm experiments were performed in a climate-controlled room at Ghent University, Marine Biology Department, Ghent (Supplementary material Figure 2.2: photo of experimental set-up). Sediment cores and in-situ seawater were transported within 24 h for subtidal samples and 5h for intertidal samples to the climate-controlled room at in situ temperature (recorded from the CTD cast on RV Simon Stevin) in the lab and immediately incubated uncapped in the open tanks filled with well-aerated in situ sea water. Each tank had a total capacity of 12 small Plexiglas corers (Ø 9 cm, height 25cm) and 4 large cores (Ø 19 cm, height 30 cm) and was filled until three quarters of the tank height to buffer for small changes in temperature and aerated by using two diffusive air stones. To prevent the organisms from escaping from the intact sediment cores, a net

(500 µm mesh size) was installed and sealed tightly on the top of each core. There was sufficient oxygen below the nets, since no obvious mortality was observed.

Macrobenthic communities were incubated in their natural sediments for 12 days at ambient temperature and salinity in well-aerated water that was collected in situ. Organisms were not fed during the incubation. Water temperature was 20, 10, 6 and 18 °C in September, December, March and June, respectively. Salinities were measured in each season: in the polyhaline zone, it varied from 30 to 24 between September and December and down to 20 in March and June; in the mesohaline zone, the corresponding values were 15, 15, 7 and 10, and in the oligohaline zone 2, 3, 0 and 1. Salinity and temperature in each tank were monitored daily and no distinct variations in salinity and temperature were shown during the incubation. A chronological overview of the 12 days lasting experimental activities can be found in Supplementary Figure 2.2. This Chapter reports the experiment-derived data on particle reworking and bio-irrigation.

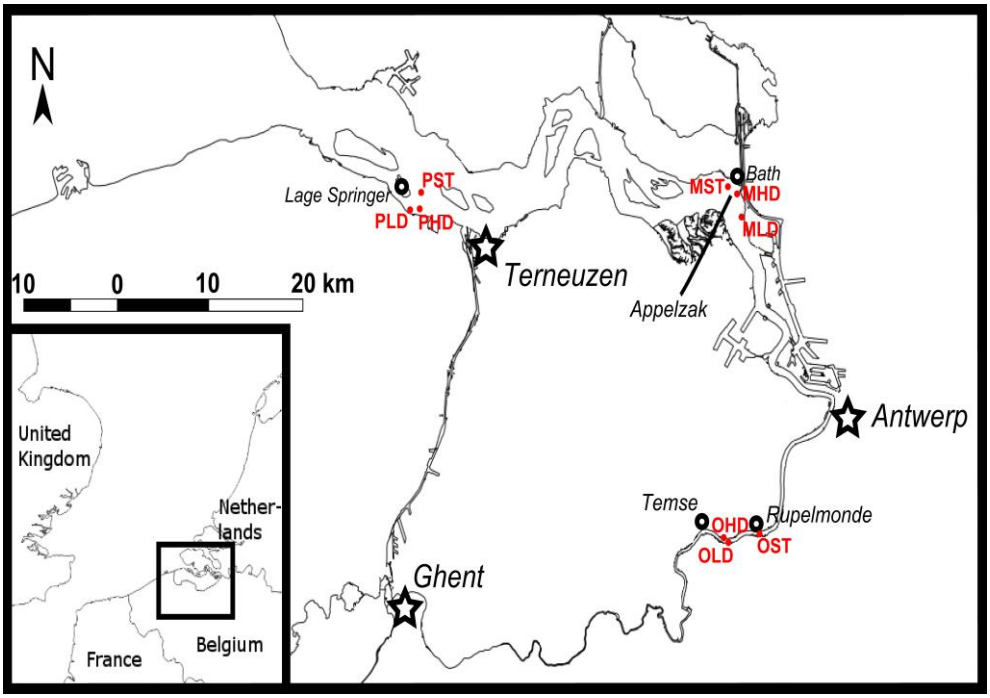


Figure 2.1: Map of the Scheldt estuary, with all sampling stations indicated. P = polyhaline, M = mesohaline, O = oligohaline, ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic

2.2.2 Quantification of particle reworking and bio-irrigation

The movement of water from the water column to the sediment was estimated from the change over time in the concentration of bromide in the water column (Forster et al. 1999). Therefore the overlying seawater was replaced by a mixture of seawater and 0.1 M NaBr (Rao and Jahnke, 2004) on the eighth day of incubation. Immediately after the tracer addition, 2-ml aliquots were sampled with pasteur pipettes and subsequently after 2, 4, 22.5 and 24 h. The samples were kept refrigerated in the dark before analysis. Relations between the Br− concentration in the overlying water column and time after addition of NaBr were determined with simple linear regression. Bio-irrigation rates were then calculated as the porewater exchange rate (in ml min^{-1}) (Meysman and Bruers, 2007) and further standardised to ml min^{-1} m^{-2}. Data from the oligohaline sites in March and from the mesohaline high-dynamic intertidal habitat in June were omitted due to measurement errors resulting from a mismatch in density between tracer solution and porewater, leading to erroneously high solute transfer rates.

Particle reworking rates were modelled from vertical profiles of redistributed luminophores (Mahaut and Graf, 1987). Two different size classes of luminophores (Environmental Tracing Systems Ltd.; colour “Magenta”, approximate median grain sizes of 120 µm for application on coarse sediments and 30 µm for fine sediments) were used. At each sampling site, the natural sediments were collected and dried for 48h at 60 °C in the oven. 1 g of 120 µm luminophores was added to 6 g of natural dried sediment and mixed homogeneously. All the organic matter in these sediments was previously removed by muffling. Subsequently, in situ seawater was gently added until a homogeneous mix was formed. The mix was poured in moulds of 9 cm diameter (= internal diameter of the small cores) and 5 mm deep. Due to practical difficulty in making

and maintaining 5 mm mud cakes to cover the surface of large cores (internal diameter 19 cm), the rectangular moulds with the length and width 6.5 cm and 13 cm were used to make mud cakes for the large cores. Two rectangular mud cakes were made for each large core. All the mud cakes were frozen at -20°C until the start of experiment. At the onset of the experiment, frozen tracer slices were placed on the sediment surface covering the entire surface of the small cores and the centre 13x13 cm surface of the large cores, and then incubated for twelve days. Such relocation in shallow coastal sediments has been observed in pulse experiments that lasted a couple of days (e.g. 48 h in Solan et al. 2004a) to weeks (e.g. 28 d in Fernandes et al. 2006). In a previous study in the Scheldt estuary a 14-day incubation time was shown successful (Mestdagh et al. 2018). We have therefore adopted a similar timing. Moreover, this time scale allowed us to sample other processes (e.g. benthic-pelagic nutrient exchange) that not are not shown in this paper (a chronological overview of the experiment can be found in **Chapter 3** Figure 3.2). As luminophores are mixed with underlying sediment by the particle mixing of the macrofauna, a measurable vertical profile is created. On the eleventh or twelfth day of the incubation, sediment communities were sliced per 0.5 cm for the uppermost 2 cm, and then per cm till a depth of 10 cm.

The sediment collected within each slice was homogenized thoroughly in a Petri dish and was photographed under UV light according to the methods described in De Backer et al. (2011). Petri dish, camera and UV lamp (365 nm peak wavelength) were placed in a fixed setup. Images of the homogenised sediments were digitally processed in Matlab for luminophore counting. Luminophore pixels were quantified by using quadratic discriminant function analysis, therefore the pixels were classified into two classes: luminophore tracer and background, based on their brightness value in the red band. Pixel counts were further converted to percentage of luminophore in each sediment slice

based on the total depth-integrated pixel counts for each size type. The depth to which luminophores were reworked was calculated from the deepest slice containing luminophores and is further referred to as maximum penetration depth. The limit of quantification was set to 1 % of the total fraction to exclude noise.

To estimate the overall tracer relocation pattern in a core due to particle reworking, the R package 'Turbo' (Soetaert and Provoost, 2017) was applied to fit the diffusive (biodiffusion coefficient) and random-walk particle reworking models (random-walk coefficient) (Meysman et al., 2003). The diffusive model is underpinned by assumptions of local (diffusive-like) particle displacements (directionally random, very frequent and small particle displacements) (Boudreau, 1986). The common equation for the diffusive model is:

$$\frac{\partial C}{\partial t} = D_b \frac{\partial^2 C}{\partial z^2} \qquad (1)$$

with C the concentration of tracers, t the time (in days), D_b the biodiffusion coefficient (in $cm^2\ d^{-1}$), and z the depth into the sediment from an origin fixed at the sediment-water interface (in cm). The continuous-time random-walk model tracks the displacement of individual particles and quantifies particle reworking with average waiting time (τ; the time a particle 'waits' in between subsequent displacements), step length (σ; the distance a particle moves in one displacement event), and direction of particle movement (Meysman et al., 2008; Wheatcroft et al., 2008). The average waiting time and step length can be modelled as stochastic variables, which follow probability distributions. We applied the random-walk model with a Poisson stepping process, combining an exponential waiting time distribution and a Gaussian step-length distribution. These distributions determine the evolution of a tracer profile over time (Maire et al., 2007; Meysman et al., 2008), from which the random-walk coefficient can be calculated as:

$$Random - walk\ coefficient = \frac{\sigma^2}{2\tau} \quad (2)$$

We fitted both models to the luminophore tracer data by starting with initial guesses of the parameters to create the initial profile, and the number of days after which the profile was measured (Soetaert and Provoost, 2017). Since our experiment was designed to examine the particle reworking by adding a pulse input at the sediment surface, and since particle relocation was followed over a short time period, we solved the model under non-steady state conditions. From these two models, the most appropriate coefficient was selected based on the principle of maximum parsimony by comparing the relative changes in sums of squares to degrees of freedom according to an F-test. The selected particle reworking coefficient is hereafter referred to as the particle reworking rate (in $cm^2\ d^{-1}$).

2.2.3 Macrofauna identification

After 11 or 12 days of incubation, the macrofauna was extracted from the remaining sediment not used for luminophore analysis, using a 0.5 mm mesh sieve. The macrofauna was preserved using a 4 % formaldehyde solution awaiting identification to the lowest practical taxonomic level (Montserrat et al., 2008). The total wet weight for each species per core was determined and converted to ash-free dry weight (AFDW) by means of species-specific conversion factors for the Scheldt estuary (Sistermans et al., 2007).

2.2.4 Data analysis

2.2.4.1 Spatio-temporal patterns of particle reworking and bio-irrigation

To assess the temporal and spatial variability of the bio-irrigation, particle reworking, and maximum penetration depth, a crossed 3-way Permutational Multivariate Analysis of Variance (PERMANOVA) design was adopted, with the fixed factors habitat (with three levels: subtidal, high-dynamic intertidal, low-dynamic intertidal), salinity (three levels:

polyhaline, mesohaline, oligohaline), and month (four levels: September, December, March, June). When a significant treatment effect was found, pairwise PERMANOVA tests were performed in order to detect differences between the levels of significant factors. The PERMANOVA tests were followed by a PERMDISP test to verify the homogeneity of multivariate dispersion (Anderson et al., 2008). Significant PERMDISP results indicate that the results of the PERMANOVA tests should be interpreted with caution. PERMDISP pointed at heterogeneity of variances in particle reworking rates and maximum penetration depth that complicated the interpretation of the significant Permanova's. The MDS plots were adopted to assess data dispersion, and the significant dispersion effects were mainly due to the lower variability among ST replicates in comparison to the intertidal habitats (Supplementary material Figure 2.3).

Principal component analysis (PCA) was performed using the PRIMER-E software package (Clarke and Gorley, 2006) to evaluate patterns of particle reworking in each mesocosm. The variables included in this multivariate analysis were the relative tracer fractions (in %) in six different depth layers (0-0.5 cm, 0.5-1 cm, 1-2 cm, 2-4 cm, 4-6 cm and beneath 6 cm), as derived from the luminophore profiles, along with maximum penetration depth and the estimated particle reworking rate. All the analysis were performed in PRIMER v6.1 (Clarke and Gorley, 2006) with PERMANOVA+ add-on (Anderson et al., 2008).

2.2.4.2 Contribution of macrofauna to bio-irrigation and selection of key bio-irrigators

Multiple regression using best-subset selection was applied to identify the macrofauna drivers (i.e. species' biomasses) of bio-irrigation at the spatial and temporal scales studied. Therefore a multiple linear regression model with k predictor variables (i.e. key

macrobenthic species' biomasses) X_1, X_2,..., X_k in predicting response variable Y (i.e. bio-irrigation at community level) can be written as:

$$Y = a_0 + a_1X_1 + a_2X_2 + \cdots + a_kX_k$$

Three types of models were calculated. First, the degree to which macrofauna biomass explained the total spatio-temporal variation in bio-irrigation was assessed. Secondly, multiple regression models were calculated per habitat type and per month in order to decrease unexplained variability related to species habitat preferences and seasonal patterns of environmental conditions that could affect macrofauna behaviour (Vaughn et al., 2007). Since macrobenthic species had very low densities at subtidal habitats, regression models were not calculated for subtidal samples. Species with significant partial regression coefficients were defined as dominant contributors to bio-irrigation. Subsequently, variation partitioning (Cormack et al., 2006) was performed by means of the *varpart()* function in the R package 'vegan' (Oksanen et al., 2017), allowing the quantification of the portion of the variation in bio-irrigation explained by the biomass of a specific species population when controlling for the effect of the other species. Bio-irrigation data for the full model (i.e. all sites and sampling moments) and the model for December were fourth-root transformed to meet the assumptions for the use of multiple regression: normality of the residuals, autocorrelation of errors, homoscedasticity, non-linearity and residual autocorrelation.

2.2.4.3 Contribution of macrofauna to particle reworking and selection of key contributors

Distance-based linear models (distLM) were used in PRIMER-E to determine to what extent the variation of the macrofauna species AFDW could explain the multivariate variation in particle reworking metrics, composed of eight parameters: luminophore fractions at the depths of 0-0.5 cm, 0.5-1 cm, 1-2 cm, 2-4 cm, 4-6 cm and beneath 6 cm,

maximum penetration depth and modelled particle reworking rate. We applied this multivariate analysis as assumptions for best-subset regressions of univariate particle reworking metrics were not met for all habitat types or months. A stepwise routine that employed 9999 permutations, based on the AICc selection criterion, was implemented to determine which model best explained variation in multi-variable sediment reworking metrics. The contribution of species population biomass was calculated for the total data set, as well as per intertidal habitat type (high-dynamic and low-dynamic) and month separately. As with the multiple linear regression models described in 2.4.2, subtidal models were not considered due to the low abundance of macrofauna.

2.3 Results

2.3.1 Spatio-temporal variability of bio-irrigation

PERMANOVA tests indicated significant differences in benthic community bio-irrigation between habitat type, salinity and month (Month*Habitat*Salinity interaction; Table 2.1a). Bio-irrigation varied between 1.47 ± 1.00 ml min^{-1} m^{-2} (oligohaline high-dynamic intertidal in December; mean ± standard error) and 10.04 ± 1.25 ml min^{-1} m^{-2} (polyhaline high-dynamic intertidal in December; mean ± standard error) (Figure 2.2a), with generally higher irrigation in the intertidal habitats of the polyhaline zone and the low-dynamic intertidal habitat in the mesohaline zone. At these habitats with high irrigation, irrigation was lowest in June, but months with maximum irrigation varied strongly. In the habitats with overall low bio-irrigation, rates generally peaked in March and/or June.

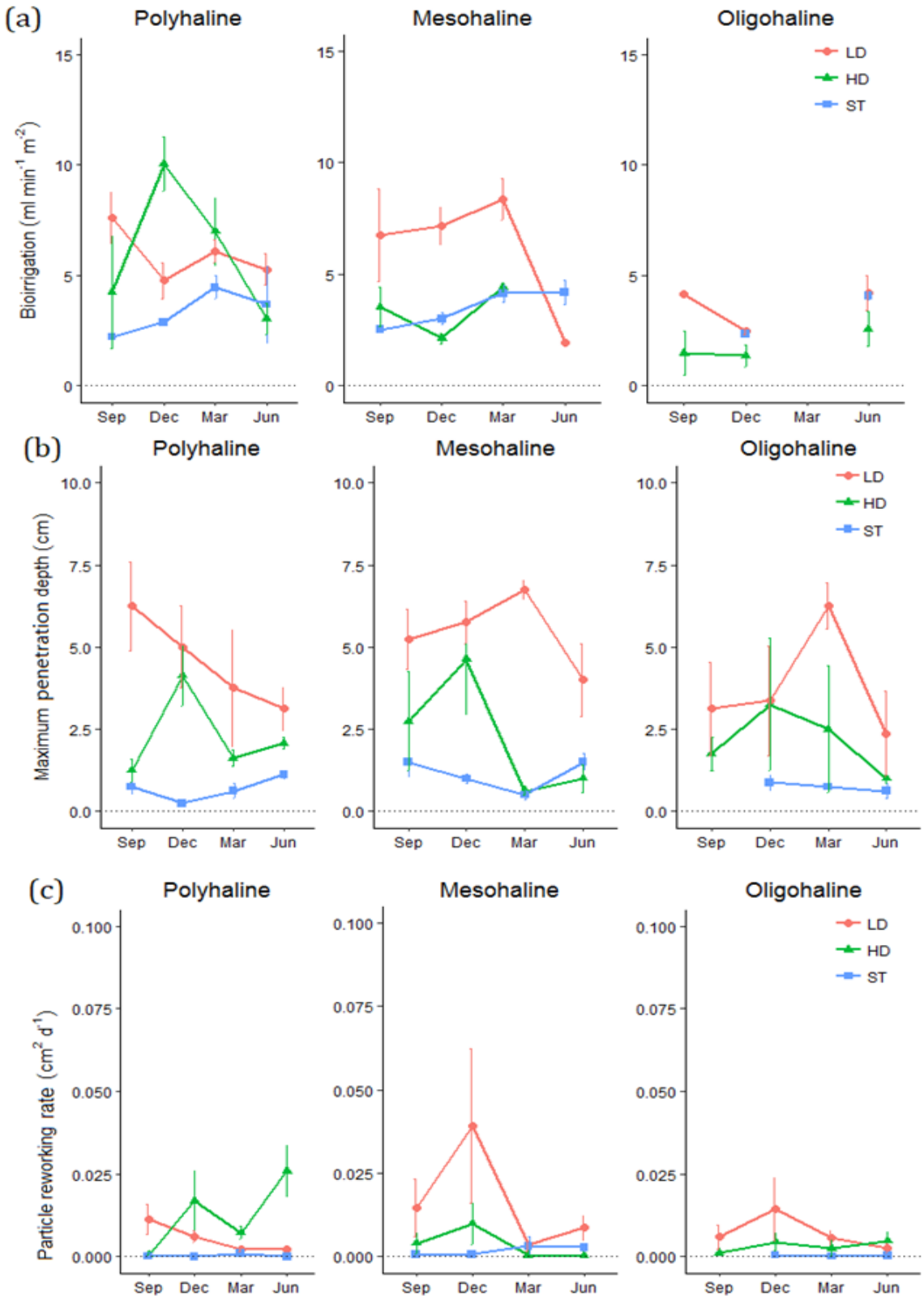


Figure 2.2: Overview of (a) bio-irrigation, (b) maximum penetration depth, and (c) particle reworking rate quantified from four replicates at nine stations in the Scheldt estuary in September/December 2015 and March/June 2016 (ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal). Error bars

represent the deviation of standard errors from the mean. Data points in each month were independent from each other.

2.3.2 Spatio-temporal variability of particle reworking

Relocation of luminophores from the sediment surface deeper into the sediment was observed at all intertidal habitats, whereas luminophores predominantly remained at the surface in subtidal habitats (Figure 2.3). Particle mixing was significantly deeper in the low-intertidal habitats except in December (Habitat*Habitat; Table 2.1a,b; Figure 2.2b). Significantly different benthic community particle reworking rates among habitat type and salinity were found in different seasons (Month*Habitat*Salinity interaction; Table 2.1a,b). Particle reworking rates varied between $2.86 \pm 1.34 \times 10^{-5}$ cm^2 d^{-1} (polyhaline subtidal in December; mean ± standard error) and $3.90 \pm 2.32 \times 10^{-2}$ cm^2 d^{-1} (mesohaline low-dynamic intertidal in December; mean ± standard error) (Figure 2.2c), and were lower in subtidal habitats as compared to both intertidal habitats, except for the mesohaline high-dynamic intertidal habitats that had lower particle reworking rates in March and June. Particle reworking rates in the intertidal habitats were highest in September and December and lowest in March, except for the polyhaline high-dynamic intertidal habitat, where particle reworking was lowest in September and peaked in June; and for the oligohaline high-dynamic intertidal habitat where little temporal variability was observed.

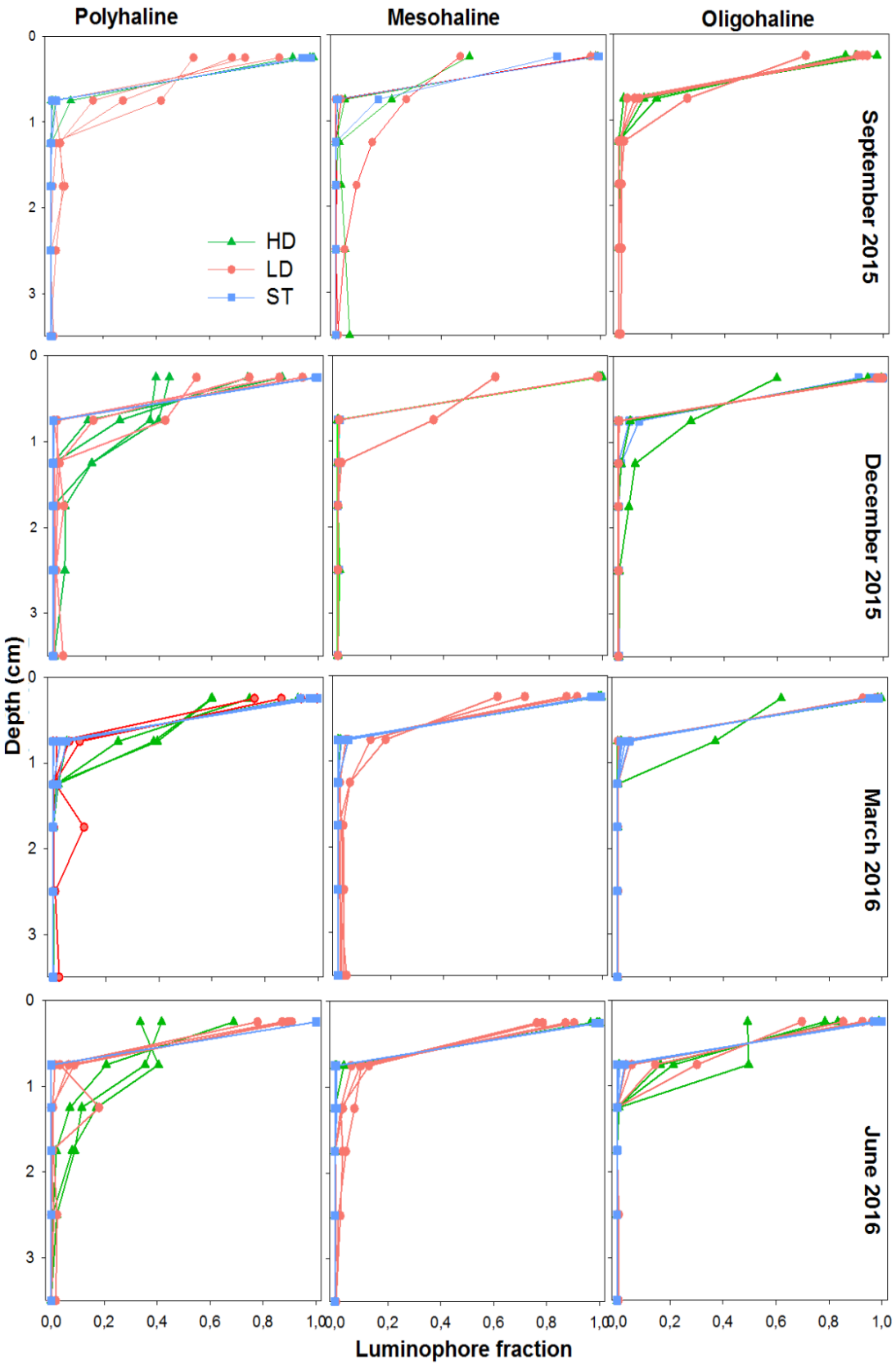


Figure 2.3: Depth profiles of luminophore concentrations for all replicates, estimated from photographs taken under UV light and given as a proportion of the total amount of luminophores in the complete core.

Only the top three centimetres are presented for a better visualization of the plots. HD = high-dynamic intertidal, LD = low-dynamic intertidal, ST = subtidal.

Table 2.1: Spatio-temporal effects on bio-irrigation, particle reworking rates and maximum penetration depth. (a) Main test of PERMANOVA and the last column shows the p-values of PERMDISP tests. Mo = Month, Sa = Salinity, Ha = Habitat. (b) Significant pair-wise differences between levels of the different factors, for which the main test was significant ($P < 0.05$). Sep = September, Dec = December, Mar = March, Jun = June, P = polyhaline, M = mesohaline, O = oligohaline, ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal.

(a) Variable	Effect	Pseudo-F	p	p (PERMDISP)
Bio-irrigation rate	Mo	4.21	0.008	
	Sa	8.44	<0.001	
	Ha	11.12	<0.001	
	MoxSa	3.00	0.014	
	MoxHa	2.97	0.008	
	SaxHa	4.48	0.001	
	MoxSaxHa	2.98	0.005	0.212
Particle reworking rate	Mo	2.77	0.043	
	Sa	1.75	0.180	
	Ha	6.75	0.002	
	MoxSa	0.68	0.638	
	MoxHa	2.86	0.012	0.023
	SaxHa	2.40	0.051	
	MoxSaxHa	1.00	0.443	
Maximum penetration depth	Mo	2.80	0.046	
	Sa	3.37	0.035	0.301

	Ha	33.28	<0.001	
	MoxSa	0.02	1.000	
	MoxHa	2.43	0.031	0.001
	SaxHa	0.83	0.516	
	MoxSaxHa	1.07	0.388	

(b)	Month	Salinity	Habitat
Bio-irrigation	MHD: Dec-Mar MST: Sep-Mar, Sep-Jun, Dec-Mar OLD: Sep-Dec OST: Dec-Jun PHD: Dec-Jun PST: Sep-Dec, Sep-Mar, Dec-Mar	SepLD: O-P DecHD: M-P, O-P DecLD: M-O, O-P DecST: O-P	SepM: LD-ST SepO: HD-LD SepP: LD-ST DecM: HD-LD, LD-ST DecP: HD-LD, LD-ST, HD-ST MarM: HD-LD, LD-ST JunO: HD-ST
Bioturbation	HD: Sep-Jun, Mar-Jun ST: Dec-Jun		Sep: HD-LD, LD-ST Dec: HD-ST, LD-ST Jun: HD-ST, LD-ST
Maximum penetration depth	HD: Dec-Mar ST: Dec-Jun, Mar-Jun	M-O	Sep: HD-LD, LD-ST Dec: HD-ST, LD-ST Mar: LD-ST, HD-LD Jun: HD-ST, LD-ST

The PCA (Figure 2.4) described the spatio-temporal variability in multivariate composition of particle reworking parameters, with the first five axes combined explaining 93.5 % of the total variation (Table 2.2). The first principal component (PC1) contributed 48.7 % to the variability and correlated positively to the variation in

luminophore fractions present in the upper 0.5 cm, mainly separating habitats with limited particle reworking (i.e. subtidal, high-dynamic intertidal habitats in the mesohaline and oligohaline zones) from the other habitats. In addition, the second PCA axis explained 19.1 % of the total variation and separated samples mainly according to depth of sediment reworking, separating deeper reworking (< 2 cm) in the low-dynamic intertidal habitat during the cold months (December and March) from the shallower reworking during warmer months (September and June).

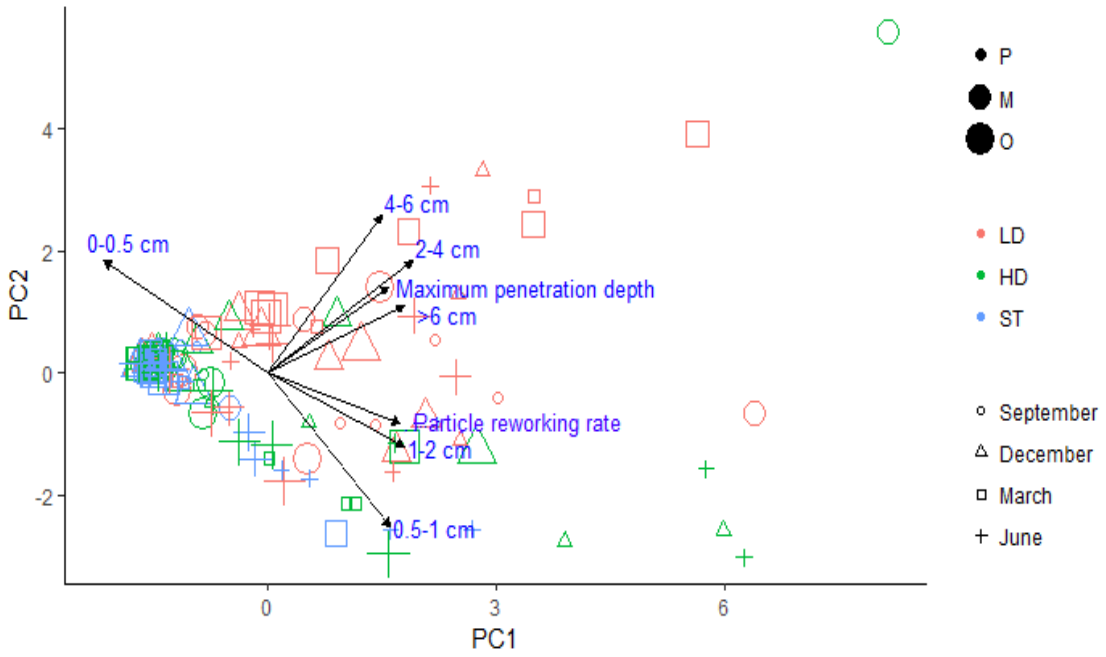


Figure 2.4: Principal component analysis (PCA) of particle reworking metrics, composed of the calculated particle reworking rate and experimentally derived luminophore fractions at depths of 0-0.5 cm, 0.5-1 cm, 1-2 cm, 2-4 cm, 4-6 cm, > 6 cm, and maximum penetration depth. P = polyhaline, M = mesohaline, O = oligohaline, LD = low-dynamic intertidal, HD = high-dynamic intertidal, ST = subtidal.

Table 2.2: Correlation-based principal component analysis (PCA) of normalised sediment reworking parameters. Eigenvalues and % variation explained by the first 5 ordination axes are given.

Variable	PC1	PC2	PC3	PC4	PC5
% Variation	48.7	19.1	11.1	8.6	6.0

Particle reworking rate	-0.35	0.16	-0.64	-0.02	0.26
Maximum penetration depth	-0.32	-0.28	-0.36	-0.67	0.004
0-0.5cm	0.43	-0.37	-0.29	0.10	0.05
0.5-1cm	-0.32	0.50	0.39	-0.33	0.01
1-2cm	-0.36	0.24	-0.26	0.52	-0.42
2-4cm	-0.38	-0.37	0.02	0.21	-0.33
4-6cm	-0.30	-0.52	0.35	-0.09	-0.23
>6cm	-0.36	-0.22	0.20	0.35	0.77

2.3.3 Identification of key bio-irrigators

In total, 28.9 % of the spatio-temporal variation in bio-irrigation was explained by the biomass of two species: *Corophium arenarium* and *Hediste diversicolor* (Model formulations refer to Supplementary materials Table 2.1). The contribution of these and other key bio-irrigators to the variability in community bio-irrigation varied strongly between seasons and habitats (Table 2.3). In the model describing the variation in low-dynamic intertidal habitats, no less than 47.6 % of the variability in bio-irrigation could be explained by the biomass of only *H. diversicolor*. A similar amount of explained variability (R^2_{adj} = 0.527) was attributed to the combined contributions of *H. diversicolor*, *Limecola balthica* and Oligochaeta spp. in the model describing the variation in the high-dynamic intertidal habitats. Overall, the predictability of bio-irrigation by macrofauna biomass was lowest in June, and the seasonal contributions of individual species to bio-irrigation were highest in September (*H. diversicolor*) and December (*C. arenarium*) (Table 2.3).

Table 2.3: The proportion of bio-irrigation variation explained by biomasses of selected species in multiple regression models (based on the full dataset or on subsets defined by months or habitats), testing bio-

irrigation versus species biomasses. R^2_{adj} values (significant level P<0.01) are given for each model. HD = high-dynamic intertidal, LD = low-dynamic intertidal (full linear regression models refer to supplementary materials of Chapter 2 Table 2.1).

	September	December*	March	June	HD	LD	All*
Corophium arenarium		0.44					0.05
Corophium volutator	0.10		0.17				
Hediste diversicolor	0.44	0.27	0.26	0.16	0.13	0.48	0.26
Limecola balthica					0.13		
Oligochaeta					0.11		
R^2_{adj}	0.61	0.66	0.51	0.16	0.53	0.48	0.29

* The data for the total model and the model in December were fourth-root transformed before analysis, to meet the assumptions for linear regression

2.3.4 Identification of key contributors in particle reworking

DistLM results showed that the variability in biomass of five species together significantly explained 21.2 % of the total spatio-temporal variation in particle reworking metrics: *Bathyporeia pilosa*, *C. arenarium*, *H. diversicolor*, *Eteone longa* and Oligochaeta spp. (Table 2.4). Predictability of particle reworking by macrofauna was lower in low-dynamic intertidal than in high-dynamic intertidal habitats, and the models based on the individual seasons had a higher predictive power than habitat models (Table 2.4). For each month, two to four species contributed significantly to the variability in particle reworking between samples. In December and March *H. diversicolor* contributed most, while *Corophium volutator* and *B. pilosa* were the species that contributed most to the

spatial variability in September and June, respectively. Other species never contributed more than 10 % to the total variation (Table 2.4).

Table 2.4: Contribution of selected species to the multi-variable sediment reworking, quantified with distance-based linear models (DistLM). R^2 values (significant level $P<0.1$) are given for each model. HD = high-dynamic intertidal, LD = low-dynamic intertidal.

	September	December	March	June	HD	LD	All
Bathyporeia pilosa				0.35	0.21		0.08
Corophium arenarium			0.09				0.02
Corophium volutator	0.28						
Eteone longa				0.06			0.02
Hediste diversicolor		0.19	0.37			0.06	0.07
Lacuna vincta				0.09		0.07	
Oligochaeta	0.10	0.07					0.03
Peringia ulvae		0.08					
Retusa obtusa			0.09	0.07			
R^2	0.52	0.34	0.55	0.55	0.21	0.13	0.21

2.4 Discussion

2.4.1 Spatio-temporal patterns of community particle reworking and bio-irrigation

We demonstrated that particle reworking and bio-irrigation in the Scheldt estuary depend on environmental conditions that structure macrobenthic communities in space and time as well as on habitat type. The patterns in macrobenthos along the salinity gradient and between habitat types (Supplementary material Figure 2.4) agree with earlier work demonstrating that species distribution and biomass variation are influenced by sediment characteristics on a local scale within the same salinity zone (e.g. Flint and Kalke 1985; Ysebaert et al. 1998; Van Colen et al. 2010b). The lower biomass in the subtidal channel as compared to the intertidal habitats can be explained by the high current velocity and associated instability of the sediment, leading to communities poor in macrobenthos (Ysebaert et al., 2002). In general, particle reworking and bio-irrigation were low in the subtidal, as well as in the low populated high-dynamic intertidal habitats in the meso- and oligohaline zones. However, bio-irrigation in June and particle reworking in March and June in those habitats often occurred at similar or even higher rates as in more densely populated habitats in the intertidal. This finding suggests that in addition to habitat structuring effects on the distribution of populations with specific sediment reworking and burrow ventilation traits, seasonal differences in these behavioural activities are also important to consider. Seasonal differences have been highlighted in previous studies that variability in community level bioturbation could be driven by the seasonal changes in seawater temperature and food availability to the benthos (e.g. Ouellette et al., 2004; Queirós et al., 2015).

Vertical luminophore profiles exhibited a typically biodiffusive pattern of particle displacement in all habitats and during each month. The particle reworking rates found

in this study are in line with the rates documented from studies using similar methods in the Scheldt estuary (Beauchard et al., 2012), the Venice Lagoon (Gerino et al., 2007) or the Plymouth Sound (Queirós et al., 2015). Indications of non-local transport were only found in some low-dynamic intertidal habitats during some months, with subsurface peaks occurring at different depths. This bio-advective transport can be explained by the high biomass of either *Heteromastus filiformis* and/or oligochaetes in these communities (Supplementary material Figure 2.4a,b). Both are subsurface deposit-feeders that convey particles vertically towards the sediment surface (Aller and Yingst, 1985; Cadée and Hegeman, 1986; Mermillod-Blondin et al., 2011). The luminophore profiles, however, showed little evidence for bio-advective transport in the oligohaline low-dynamic intertidal habitat, in which the community was dominated by oligochaetes. Consequently, we conclude that the bio-advective transport during oligochaete feeding must be overruled by the biodiffusive particle displacement during movement. Particle reworking at this location occurred deeper in March when densities (16457 ± 1183 ind m^{-2}; mean ± standard error) were 2-5 times higher as compared to the other months, corroborating evidence of movement to deeper strata at high densities (McCall and Fisher 1980; Robbins et al. 1979). Finally, our PCA analysis revealed that in addition to mixing rates, the depth to which particles are displaced is also instrumental in explaining variability in particle reworking in space and time, e.g. deeper reworking of particles in the low-dynamic intertidal habitat and deepest reworking during the coldest months in the high-dynamic intertidal habitat (December) and in the low-dynamic intertidal habitats in the meso- and oligohaline zones (March).

2.4.2 Spatial and temporal contribution of macrobenthos species to particle reworking and bio-irrigation

The predictability of particle reworking and bio-irrigation by the specific macrobenthos species biomasses varied temporally and between habitat types. Different combinations of species predicted these benthic processes in different seasons or habitats. Macrobenthos activity often depends on factors such as temperature, biotic interactions, salinity, food resource availability and quality (Akberali 1978; Ouellette et al. 2004; Alexander et al. 2015; Verdelhos et al. 2015). For example, with colder temperatures, *H. diversicolor* is known to burrow deeper into the sediment (Esselink and Zwarts 1989), which in our study was reflected by its selection as the most important contributor in particle mixing in December and March. Particle reworking rates in the polyhaline low-dynamic habitat decreased along with the overall decline in biomass of *H. diversicolor* during the study period, while biomass of the well-known bioturbators *Peringia ulvae*, *H. filiformis* and *L. balthica* simultaneously increased (Supplementary material Figure 2.4c-e). However, the selection of *H. diversicolor* as the major contributor to particle dynamics in this habitat, suggests that gallery-biodiffusion by *H. diversicolor* generally greatly outweighs the biodiffusion by other species, particularly in June, when juvenile recruits constitute the majority of the populations (Van Colen et al., 2008; Ysebaert, 2000). In the same habitat, porewater exchange rate also followed the same pattern as the biomass of *H. diversicolor*. Along with the selection of this species in all models predicting bio-irrigation, this demonstrates the coupling between particle reworking and burrow ventilation by the activity of this species (Banta et al. 1999; Palomo and Iribarne 2000; Pelegrí and Blackburn 1995). However, both processes did not follow similar temporal patterns in any of the other habitats (Figure 2.2), which suggests that the use of particle reworking as an umbrella term (Kristensen et al., 2012) to understand how particle

reworking and burrow ventilation varies in space and time is only useful for communities in which both processes are largely steered by the same species.

The predictive models for bio-irrigation had low explanatory power in June, despite high biomass of several species, including *C. volutator* (Supplementary material Figure 2.4f), which is a key irrigator of tidal flat sediments (Pelegri et al., 1994; Riisgård et al., 2007). According to Aller's transport-reaction model, irrigators will build much shorter burrows – or none at all – when the space to build burrows becomes limiting, which results in a drastic decrease of the irrigated sediment volume, both on a per capita basis and in absolute numbers (Aller, 2004). Consequently, density-dependent suppression might explain the low predictive power of our linear model for periods when densities of irrigators were high.

Finally, the contributions of specific populations to particle reworking and bio-irrigation might simply relate to the dynamic nature of intertidal flats, where dispersal and recruitment strongly influence temporal community dynamics (Van Colen et al. 2010a; Menge and Sutherland 1987). Such processes seemed to be particularly important in the polyhaline high-dynamic intertidal habitat, which is richer in species in comparison to the same habitat types further upstream. At this location, temporal dynamics in bio-irrigation rates corresponded to the (dis)appearance of populations of *C. arenarium* and *E. longa* (Supplementary material Figure 2.4g,h), of which at least *C. arenarium* is a known bio-irrigator (Gamble, 1970). Similarly, dynamics in particle reworking rates in this habitat corresponded to the (dis)appearance of populations of *P. ulvae* and *B. pilosa* (Supplementary material Figure 2.4d,i).

2.4.3 The role of bioturbation in a wider context

The findings of this chapter, together with other studies highlight that the loss of species and change of habitats will affect ecosystem processes and ecosystem functioning in soft-

sediment ecosystems (Solan et al., 2004a; Braeckman et al., 2014b). For example, anthropogenic disturbances such as dredging and dumping, or changes in nutrient loading would not only affect benthic and pelagic habitats (Hibma et al., 2008; Jeuken and Wang, 2010; Lohrer and Wetz, 2003; Mestdagh et al., 2018b), but also the biota inhabiting the seabed. Besides loss of organisms, habitat change will affect the role benthic biodiversity plays in ecosystem functioning. Chapter 3 shows that benthic macrofauna in particular affect biogeochemistry in low dynamic intertidal habitats that are characterized by low permeability and a high standing stock of organic matter. Loss of species in these habitats will thus affect the cycling of nutrients in estuarine ecosystems with likely implications for e.g. marine and estuarine food webs. Furthermore, deposition of sediments and high concentrations of suspended solids associated with dredging activities can alter the behavioural activities of benthic macrofauna with cascading effects on biogeochemical cycling (Mestdagh et al. 2018a,b). The IPCC report on climate change (IPCC Climate Change 2014) predict e.g. temperature-driven changes in sea level and the frequency of storms. This is expected to increase the loss of macrobenthos-rich intertidal habitats due to coastal squeeze in areas where migration of habitats is blocked by sea defences like the dikes along the Scheldt estuary. Finally, global warming will affect how benthic animals contribute to biogeochemical cycling, through direct effects on respiration (Yvon-Durocher et al., 2012) and indirect effects related to the activities of macrobenthos (Ouellette et al., 2004; also in this Chapter).

2.5 Conclusion

We showed that particle reworking and bio-irrigation in the Scheldt estuary vary in space and time, related to biomass-dependent effects of different species, thereby confirming our first hypothesis. Furthermore, the ephemeral presence of populations in high-dynamic habitats, density-dependent suppression in burrow ventilation and seasonal

variations in burrowing depth are suggested to explain the observation that species contribute differently to both processes in space and time, supporting our second hypothesis. Finally, *H. diversicolor* appears to be a key species for both the displacement of sediment particles and the irrigation of intertidal sediments, demonstrating its pivotal role for ecosystem functioning in these habitats.

Acknowledgements

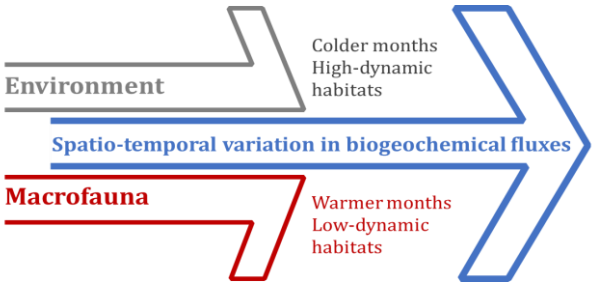
This work was co-funded through a MARES Grant (2012-1720/001-001-EMJD), a Joint Doctorate program selected under Erasmus Mundus coordinated by Ghent University (FPA 2011e0016). Sebastiaan Mestdagh acknowledges the Special Research Fund of Ghent University for a PhD fellowship (BOF.DOC.2014.0081.01). The authors wish to thank the crew of *RV Simon Stevin* for their assistance during the subtidal sampling campaign. The laboratory staffs Dirk Van Gansbeke and Bart Beuselinck at the Marine Biology Research Group of Ghent University, and Jan Sinke and Peter Van Breugel at NIOZ (Royal Netherlands Institute for Sea Research) are thanked for their contributions in analyses of samples. The colleagues at Marine Biology Group of Ghent University Guy Desmet, Mohammed Alsebai, Katja Guilini, Christophe Verwimp, Annick Van Kenhove, Niels Viaene, Elise Toussaint, Emil De Borger, Ninon Mavraki, Ee Zin Ong and Thomas Bauwens are gratefully acknowledged for their help in sampling campaigns or laboratory experiments. Many thanks also to two anonymous reviewers who helped greatly in focusing the paper. This work was supported by data & infrastructure provided by the Flanders Marine Institute (VLIZ) and the results presented in this publication were achieved using infrastructure funded by EMBRC Belgium – FWO project GOH3817N.

Chapter 3 Environmental context determines the contribution of macrofauna to benthic ecosystem functioning along estuarine gradients

Abstract

We investigated the relative contributions of macrofauna and the abiotic environment to the spatial and temporal variability in benthic biogeochemistry in the Scheldt estuary (Belgium, the Netherlands). Intact sediment communities were collected in the three main salinity zones (polyhaline, mesohaline, oligohaline) and in three habitat types (subtidal, high-dynamic intertidal, low-dynamic intertidal) in each salinity zone in four consecutive seasons in 2015 and 2016. Fluxes of oxygen, nitrate, nitrite, ammonium and phosphate were measured, as well as environmental properties of the sediment and water, macrofaunal activity (particle reworking, particle penetration depth, and bio-irrigation rates) and macrofauna sediment reworking trait composition. Using Distance based linear modelling, we found that macrofaunal total density, bio-irrigation rate, and temperature collectively explained 23% of the total spatio-temporal variation in benthic biogeochemistry, with macrofauna having a higher (18%) explanatory contribution than environmental variables (5%). Macrofauna contributed particularly more than environmental conditions to variation in benthic biogeochemistry at our sites in June, September, December, while environmental conditions contributed most in March. Biodiffusors and downward conveyer feeders were selected as particular important functional groups in affecting benthic biogeochemistry. When habitat types were analysed separately, variation in temperature was always found the best predictor for variation in benthic biogeochemistry across sampling months, with macrofauna only having an explanatory contribution in low-dynamic intertidal habitats. In this habitat

type, macrofauna and environmental properties collectively explained 60 % of the total variation in benthic biogeochemistry. Our results demonstrate the importance of the abiotic environment and macrofauna communities in controlling ecosystem functioning, highlighting that loss of biodiversity and environmental changes will affect ecosystem functioning in estuarine sediments.



Graphical abstract of Chapter 3

3.1 Introduction

Functional traits of macrobenthos play an important role in structuring the biogeochemical fluxes in soft-sediment benthic ecosystems (Kristensen et al., 2014; Thrush et al., 2006). Research on the relationships between functional traits and biogeochemistry is increasingly relevant, since a wide array of (mostly anthropogenic) environmental changes have altered the functional composition of many benthic communities, with potential effects on the benthic biogeochemistry (Levin et al. 2009). Biogeochemical cycling in marine and estuarine ecosystems is affected by the cycling of oxygen and nutrients across the sediment-water interface (i.e. benthic-pelagic exchange), supporting food web energy cycling (Griffiths et al., 2017). Benthic-pelagic coupling depends heavily on macrofaunal abundance and biomass, and on functional traits such as burrow ventilation and sediment reworking (Norkko et al., 2015; Pratt et al., 2015; Van Colen et al., 2012), which increase the exchange surface and affect the microbial communities that mainly control the benthic biogeochemistry (Belley and Snelgrove,

2016; Foshtomi et al., 2015; Kristensen and Hansen, 1999). The functional identities of the macrofauna are often found to be more important to explain patterns in biogeochemical cycling than taxonomical diversity (Belley and Snelgrove 2017; Braeckman et al. 2014a).

Environmental conditions in estuarine systems are highly variable in space and time. Estuaries harbour a relatively large number of different habitats, caused by their transitional character between the terrestrial and marine environment, and between deep channels and intertidal flats. Seasonal variability in weather patterns causes an additional variability in abiotic conditions (Ysebaert and Herman, 2002). This abiotic variation is an essential factor determining the spatio-temporal distribution of many organisms, including the macrobenthos (Peterson and Ross, 1991; Ysebaert et al., 2000, 1993), but also influences the heterogeneity of biogeochemical fluxes (Eriksson et al., 2003; Kauppi et al., 2017). Differences in the abundances of species with specific functional traits and their seasonal patterns in behaviour, may also directly affect ecosystem functioning (Wohlgemuth et al., 2017). Biotic contributions to ecosystem functioning in estuaries are thus expected to be highly variable and context-dependent.

The estuary of the river Scheldt is a temperate, macrotidal estuary in north-western Europe (Belgium, the Netherlands). It is characterised by a full salinity gradient, including a large brackish to freshwater tidal zone, and it is home to a diverse array of habitats, ranging from tidal marshes over low- or high-dynamic intertidal flats and shoals, to shallow and deep subtidal parts (Meire et al., 2005). The spatial variability of macrobenthic communities is well studied, both along the salinity and the tidal elevation gradient (Van Colen et al., 2010a; Ysebaert et al., 2003), and their temporal variation is also well established (Ysebaert and Herman, 2002). The estuary is currently recovering from excessive nutrient loadings during the past decades, but is still characterised as

eutrophic (Meire et al. 2005; Soetaert et al. 2006; Kromkamp and Van Engeland 2010). Long-term changes in water-column biogeochemistry are thoroughly documented in the Scheldt, but much less is known about the sediment biogeochemical processes or the biogeochemical fluxes between the sediment and the water column. Furthermore, the relationships between the spatio-temporal variation of estuarine benthic solute fluxes and macrobenthos functional traits have been understudied worldwide. This paper is the first study in which such relationships are assessed along different environmental gradients in the Scheldt estuary. We quantify the contributions of macrofaunal and environmental determinants to the biogeochemical cycling in different habitats along the horizontal (i.e. salinity) and vertical (i.e. water depth) axes of the estuary, and in different seasons. We hypothesise that processes linked to macrobenthos functional traits overrule environmental steering in periods when biotic activity is higher. Additionally, macrobenthos is expected to play a smaller role in habitats with coarser sediments, that have lower organic matter content as compared to more sheltered, organically enriched habitats (Snelgrove and Butman, 1994; Ysebaert et al., 2003).

3.2 Materials and methods

3.2.1 Sampling sites

Sampling sites were chosen along the Scheldt estuary, according to salinity and habitat (Figure 3.1). In each of the three major salinity zones (polyhaline - P, mesohaline - M, oligohaline - O), the benthic communities from three different habitats were sampled (subtidal - ST, high-dynamic intertidal - HD, low-dynamic intertidal – LD; Twisk 2002). Within each salinity zone, the locations were selected based on their proximity to each other (ensuring similar water column properties, i.e. salinity, temperature and oxygen concentration) and using ecological information obtained from ecotope maps (Arcadis, 2014; Van Ryckegem et al., 2014). The ecotope mapping in the Scheldt estuary is based

on information on salinity, emersion time or water depth, hydrodynamics, and sediment characteristics. The differentiation between high- and low-dynamic ecotopes is based on (modelled) current velocity and geomorphological information, with low-dynamic areas typically found in more sheltered conditions, often coinciding with a higher mud and organic matter content of the sediment (Figure 3.3; Twisk, 2002; van der Wal et al., 2017). The sampled subtidal habitats were all high-dynamic, since low-dynamic habitats were located too close to the shore to safely sample with a research vessel. Average sediment characteristics of all sampled locations can be found in supplementary material Table 3.1.

Subtidal samples were collected on board *RV Simon Stevin* (VLIZ – Flemish Marine Institute), and intertidal samples were collected during low tide when the flats were accessible on foot. The sampling campaigns were performed on 1-3 September 2015, 8-10 December 2015, 1-3 March 2016, and 21-23 June 2016.

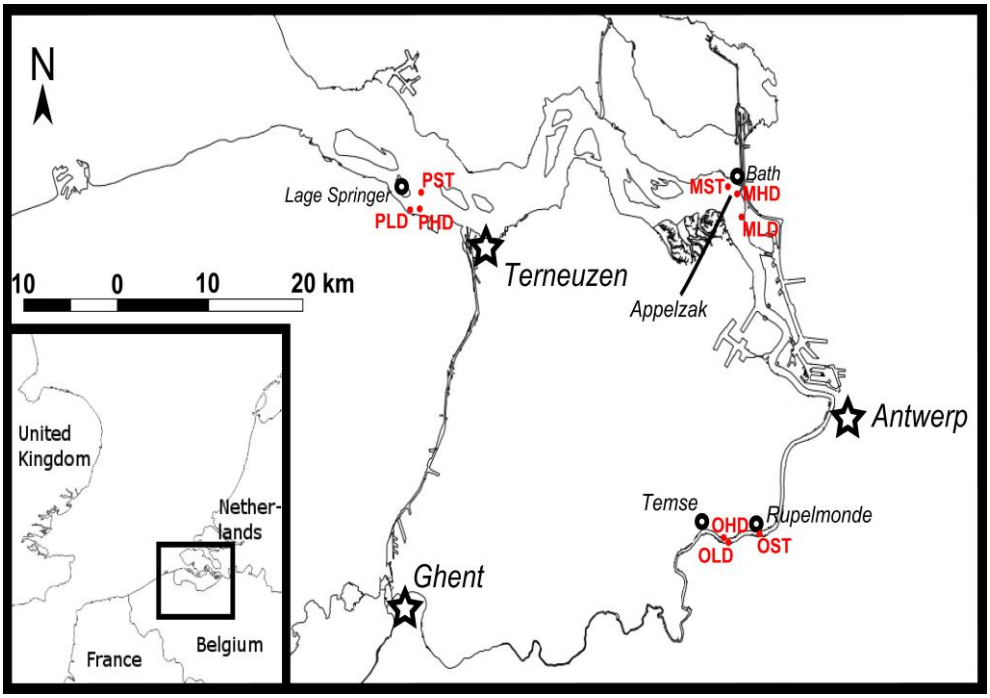


Figure 3.1: The sampling stations in the Scheldt estuary. P = Polyhaline, M = mesohaline, O = oligohaline, ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal.

Polyhaline

In the polyhaline salinity zone, samples were collected on the Paulina intertidal flat on the southern bank of the estuary, west of the Dutch city of Terneuzen. This tidal flat comprises a variety of habitats, including a salt marsh, and sediment types, from muddy to sandy almost devoid of silt (Gallucci et al., 2005). A high-dynamic intertidal location was chosen (51° 21' 00.2" N, 3° 43' 54.9" E) in a sandy area on the intertidal flat. The low-dynamic intertidal location was situated in a muddy area close to the tidal marsh (51° 20' 57.1" N, 3° 43' 35.4" E). Subtidal samples were collected right in front of the intertidal flat (51° 22' N, 3° 44' E), east of the Lage Springer shoal. The relatively high mud content in the subtidal location (Supplementary material Table 3.1) was due to the presence of small clay clods in the samples.

Mesohaline

The mesohaline intertidal samples were collected on the northern bank of the estuary, near the Belgian-Dutch border. The high-dynamic intertidal site was located near the Bath drainage sluice, in a sandy area (51° 23' 15.9" N, 4° 14' 03.2" E). Samples from a low-dynamic intertidal habitat were gathered on the Groot Buitenschoor intertidal flat, on the Belgian-Dutch border (51° 22' 29.6" N, 4° 14' 34.9" E). Subtidal samples were collected at the Appelzak, a bend of the estuary (51° 23' 26.7", N 4° 12' 59.3" E).

Oligohaline

In the oligohaline zone, two intertidal flats on either side of the estuary were chosen near the Belgian town of Temse. A high-dynamic intertidal site was found on the left bank, on the Ballooi intertidal flat (51° 07' 00.1" N, 4° 14' 48.2" E), and a low-dynamic intertidal site on the Notelaer mudflat, on the right bank (51° 06' 52.5" N, 4° 15' 24.3" E). Due to difficult access of the research vessel to the higher reaches of the estuary, a more

downstream location in the same salinity zone had to be chosen for the subtidal sampling, near the town of Rupelmonde (51° 07.5' N, 4° 18.2' E, Figure 3.1). This location was not sampled during the first sampling campaign in September 2015.

3.2.2 Sample collection and experimental set-up

On each location we collected 10 cm deep sediment samples with four Plexiglas corers. We either used small cores with magnetic stirring rods (Ø 9 cm, height 25 cm) or large corers with stirring discs (Ø 19 cm, height 30 cm), in order to allow the experimental set-up to create a sufficient pressure gradient to ensure advective water flows through the sediment (Glud et al., 2006; Huettel and Gust, 1992). Two types of corers were selected based on the perceived permeability when the sediment was sampled during the first sampling campaign in September. Coarse sediment, with high permeability, was sampled with the larger corers, while the smaller corers were used for finer, less permeable sediment. Eventually, only the high-dynamic intertidal habitats of the polyhaline and oligohaline zones were sampled with the large corers. The subtidal sites were sampled with a 0.25 m^2 NIOZ box corer, in which small Plexiglas corers were inserted, one corer per box core sample. In the laboratory, a frozen mud cake was added on the surface of all cores to estimate particle reworking rate. This mud cake was composed of a mixture of defaunated sediment and luminophores (Environmental Tracing Systems Ltd.; colour "Magenta", approximate median grain sizes of 120 µm for application on coarse sediments and 30 µm for fine sediments), in a 6:1 volume:volume ratio. All mud cakes had a thickness of 0.5 cm, and covered the entire surface of the small cores, and the centre 13x13 cm surface of the large cores. After luminophore addition, the cores were incubated at ambient temperature and salinity, in well-aerated water that was collected *in situ*. The incubation temperatures were 20 °C in September 2015, 10 °C in December 2015, 6 °C in March 2016, and 18 °C in June 2016. Salinity in each zone varied between

the seasons: 20-30 in the polyhaline zone, 7-15 in the mesohaline zone, and 0-3 in the oligohaline zone, all of which had lowest salinities in March. A chronological overview of the experiment scheme can be found in Figure 3.2.

3.2.3 Biogeochemical cycling

On the sixth and seventh days of the incubation (Figure 3.2), ecosystem metabolism and elemental cycling was measured by measuring sediment-water oxygen fluxes and nutrient exchange rates during closed incubations in the dark, by taking five subsequent 50 ml samples from the overlying water of each core at one to one-and-a-half hour intervals, after closing the cores air-tight and ensuring a constant flow inside the water column (e.g. Braeckman et al. 2014b). Of the sampled 50 ml, 20 ml was filtered over a Whatman GF/C filter (1.2 μm pore size), and immediately stored at -20°C for later nutrient analysis, while the remaining 30 ml was used to measure oxygen concentrations with a Unisense OX100 electrode. The nutrient samples were analysed for nitrogen components (nitrate, nitrite, ammonium) and phosphate via Continuous Flow Analysis (SAN++, Skalar, Breda, the Netherlands). These data were used to estimate oxygen and nutrient fluxes by fitting a linear model of the concentration as a function of time and taking into account the water volume above the sediment.

3.2.4 Benthic activity and identity, and sediment characteristics

After the oxygen and nutrient flux measurements, the overlying water of the cores was removed and replaced by a solution of bromide (Br-) in seawater on the eighth day of the incubation (Figure 3.2). The Br- solution was prepared with the same density as the seawater, with which it was then mixed to achieve a final bromide concentration of 0.01 M, and aerated for one night. After the addition of the new seawater solution, 2 ml water samples were taken over a total period of 24 hours; at three instances with one hour in between on day 8 and twice on day 9. The cores were aerated throughout the sampling

period. Samples were kept at 4°C before analysis with ion-chromatography. The Br- concentrations were used to calculate a bio-irrigation coefficient Q (in ml min^{-1} m^{-2}; Meysman et al. 2007) based on the measured decrease in Br- concentration. A close inspection of the bio-irrigation data revealed that some observations had to be removed due to methodological errors, which appeared to be extreme outliers (Cook's distance). No reliable data could be produced for the oligohaline zone in March. Probably, the low salinity (fresh water) caused a mismatch in density between the pore water and the bromide-seawater mix, creating unrealistically high bio-irrigation rates. In addition, the mesohaline high-dynamic intertidal habitats in June showed abnormally high bio-irrigation rates. These values might have been caused by the proximity of the drainage sluice in Bath, that regularly releases fresh water into the estuary. Since the seawater we used was collected at the subtidal site further offshore, a similar density mismatch as in the March samples might have occurred.

The luminophores, added as a frozen mud cake on the sediment surface, served as a tracer to measure particle mixing in the core. As luminophores are mixed with underlying sediment by the bioturbating action of the macrofauna, a measurable vertical profile is created. On the eleventh and twelfth day of the incubation, all cores were sliced in 0.5 cm for the first four slices, and 1 cm thick slices further down. The larger cores were subsampled in the centre with a Ø 9 cm corer. Each slice was thoroughly homogenised and a 5 ml subsample was taken and subsequently dried for 48 h at 60°C for analysis, following the procedure described by De Backer et al. (2011). The sediment collected within each layer was homogenised thoroughly in a Petri dish (55 mm inner diameter), and pictures of a fixed surface area were taken under UV light under standardised conditions. Petri dish, camera and UV lamp (365 nm peak wavelength) were placed in a fixed setup. Pictures were taken with a digital mirror-reflex camera, Canon EOS 350D;

aperture 1/8, shutter time 1s, ISO 400, manual focus and 46 mm focal length. Images were digitally processed in Matlab v8.1 (MathWorks Inc., 2013) to count luminophore pixels. Luminophore pixel counts of both size types were then converted to percentage of tracer in each sediment slice based on the total depth-integrated pixel counts foreach size type. Based on the luminophore depth profile, a particle reworking constant (cm^2 d^{-1}) was calculated with the R package 'Turbo' (Soetaert and Provoost, 2017; see **Chapter 2**). The depth to which luminophores were reworked was calculated from the deepest slice containing luminophores and is further referred to as maximum penetration depth. The limit of quantification was set to 1 % of the total fraction to exclude noise.

Of the remaining sediment, 5 ml was taken from the first two slices for chlorophyll a analysis through high performance liquid chromatography (HPLC), 2 ml from each slice for analysis of total organic carbon (TOC) and nitrogen (TN) (FLASH 200 NC Analyzer, Thermo Scientific, Wilmington, DE, United States), and a final 30 ml from the homogenised entire sample for analysis of sediment grain size and mud content (Mastersizer 2000 particle analyser, Malvern Panalytical, Malvern, United Kingdom). The remaining sediment of all slices was collected and sieved over a sieve with a 0.5 mm mesh size, to extract the macrofauna. The fauna samples were fixed with a 4 % formaldehyde solution and stained with Rose Bengal dye to facilitate identification. All macrofauna was identified, counted and weighed, and assigned to functional groups according to Queirós et al. (2013). Four functional groups were defined according to their mode of sediment reworking, which resulted in four distinct groups (surficial modifiers, biodiffusors, upward conveyors, downward conveyors). Surficial modifiers are defined as those animals that limit their reworking activity to the topmost centimetres of the sediment column. Biodiffusors are animals whose activities result in random local mixing of sediment, while upward and downward conveyors live vertically in the sediment, facing

head down or up and transporting sediment upward or downward, respectively (Biles et al., 2003; Kristensen et al., 2012). The blotted wet weight was converted to ash-free dry weight by means of conversion factors identified by Sistermans et al. (2006).

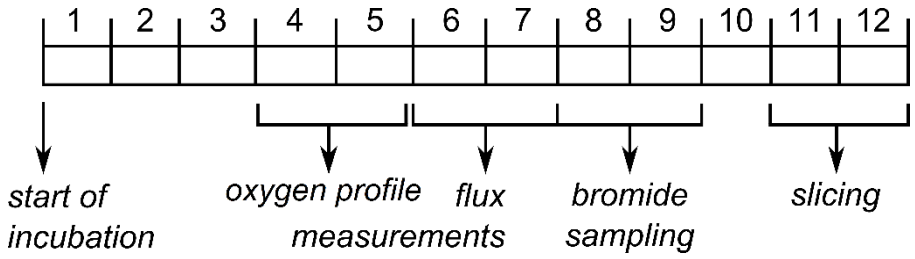


Figure 3.2: Chronological overview of the experiment, with the days represented as separate blocks. Incubation started at the beginning of day 1, followed by an acclimatisation period before the experimental measurements.

3.2.5 Statistical analysis

We examined the spatio-temporal variability in sediment properties using a centred Principal Component Analysis (PCA) on the sediment properties (median grain size, mud content, chlorophyll a concentration, total nitrogen, total organic carbon). Draftman plots verified that variables were not highly correlated (< 0.95) and all variables were thus retained in the analysis.

Non-metric multidimensional scaling (nMDS, Clarke and Gorley 2006) on the Bray–Curtis similarity index was used to illustrate spatial and temporal patterns in macrofaunal community compositions along estuarine gradients. Data of species density was square root transformed to bring all taxa to a similar relative scale of abundance and therefore increase the contribution of rare species (Anderson et al., 2008). Furthermore, vector overlays based on spearman rank correlations were employed to visualize the correlations between sediment reworking trait densities, and measured properties

related to the activity of the sediment community (particle mixing rate, bio-irrigation rate, particle mixing depth) and the spatio-temporal variability in community structure.

A three-way crossed PERMANOVA performed with 9999 random permutations was employed to analyse the differences between the fixed factors month (four levels), salinity zone (three levels) and habitat (three levels), were tested with model on sediment-water fluxes of oxygen, nitrate, nitrite, ammonium, and phosphate). We calculated the resemblance matrix from Euclidean distances of flux data, followed by pair-wise tests to further investigate the observed differences in case of significant interaction of factors. Homogeneity of multivariate dispersion was tested with PERMDISP for any of the significant terms in Permanova. The PERMDISP routine revealed heterogeneity of data dispersions, indicating that the observed patterns in fluxes can be a result of both site and dispersion (Supplementary material Figure 3.1 a-d).

Finally, the relative importance of environmental factors (median grain size, mud content, chlorophyll a concentration, total nitrogen, total organic carbon; categorical variable: temperature) and biological factors (total density, total ash-free dry weight (AFDW), particle reworking and bio-irrigation rates, maximum penetration depth of luminophores, and density and AFDW of the four functional sediment reworking groups) in predicting benthic flux variation of oxygen, nitrite, nitrate, ammonium and phosphate) was investigated using the distance-based linear model (DistLM) (Anderson and others 2008). Ammonia was cube root transformed to produce a symmetric distribution for the analysis. Euclidean distance was used to construct a single matrix of dissimilarities between samples. A stepwise routine that employed 9999 permutations, based on the AICc selection criterion, was implemented to determine which model best explained variation in biogeochemical fluxes. Sediment cores with erroneous bio-irrigation data were not included in the DistLM models.

Draftsman plots and correlation analyses (Supplementary material Figure 3.2 a,b) indicated none of the predictor variable's |r| values exceeding the recommended 0.95 correlation threshold (Anderson et al., 2008; Clarke and Gorley, 2006). Therefore, all the variables were retained in the initial fitting of DistLM. Nevertheless, strong correlations were revealed between total AFDW and surficial modifier AFDW (|r|>0.75) (Supplementary material Figure 3.2 b), in case these highly correlated predictors were selected into the same model, the one resulting in the best fit (R^2) was chosen. Eventually, total AFDW was selected in the low-dynamic model instead of surficial modifier AFDW.

We completed PCA, nMDS and DistLM analyses in PRIMER 6 (Clarke and Gorley, 2006) with the PERMANOVA+ add-on (Anderson et al., 2008). A significance level of $p<0.05$ was used in all tests. PCA and nMDS were based on the mean value per sampling location for each season (n=4), while all replicates were considered for DistLM.

3.3 Results

3.3.1 Spatio-temporal effects

3.3.1.1 Environmental variables

PCA on environmental data (Figure 3.3) highlights two main axes. Axis one separates the sediment characteristics (i.e. median grain size, total organic carbon in low-dynamic habitats from high-dynamic and subtidal habitats (Figure 3.3). The second axis is characterized by variations in Chlorophyll a and total nitrogen, exhibiting the influence of seasonal variations (e.g. temperature and food availability).

The lowest chlorophyll *a* concentrations were found in subtidal habitats throughout the first three seasons (September, December, March), followed in those habitats by peaks in June in all salinity zones. Similar summer peaks (in September and June) were observed

for the polyhaline high-dynamic intertidal habitat (Supplementary material Table 3.1). Median grain size and mud content often showed opposite patterns, in which low-dynamic intertidal habitats had highest mud contents, but lowest median grain sizes. The granulometry of subtidal habitats was especially unstable, with a peak in mud content in the polyhaline zone in December (and corresponding low median grain size), and a low mud content (with corresponding peak in median grain size) in the mesohaline zone in March (Supplementary material Table 3.1). The TN content in low-dynamic intertidal habitats was generally highest, with the exception of peaks in March of the mesohaline subtidal and oligohaline high-dynamic intertidal habitats. The TOC content was always highest in low-dynamic intertidal habitats and lowest in the high-dynamic intertidal habitats, except in March in the oligohaline zone, where the TOC of the subtidal habitat was lower (Supplementary material Table 3.1).

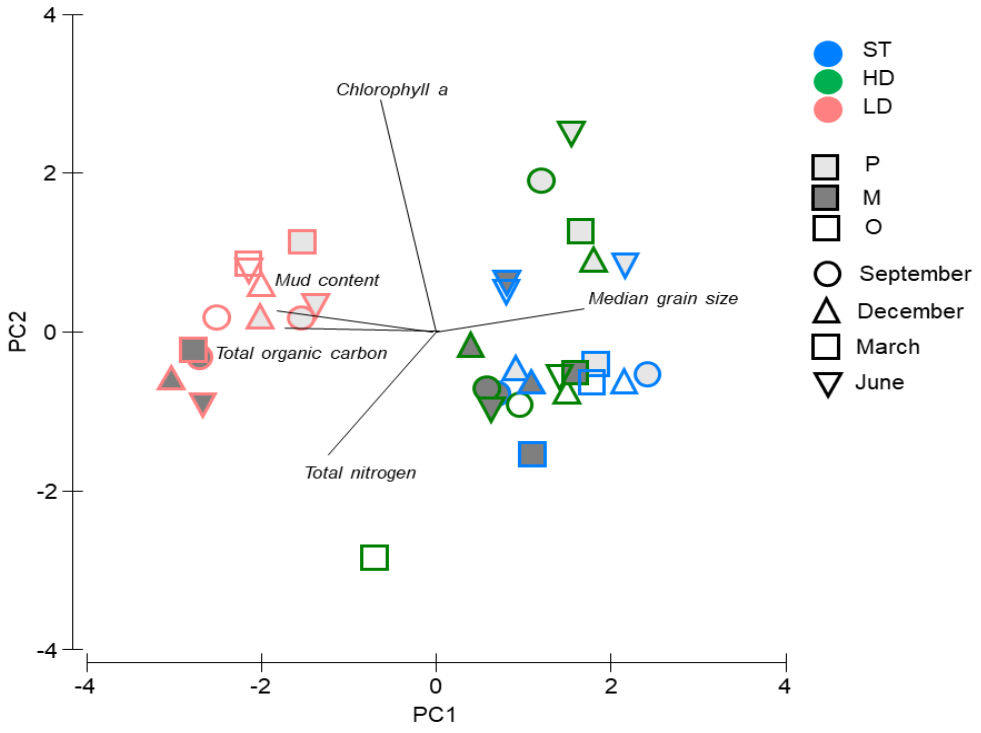


Figure 3.3: Two-dimensional PCA ordination of the environmental variables (median grain size, mud content, chlorophyll a concentration, total nitrogen, total organic carbon) for the 9 sampling sites along the

estuary. P = polyhaline, M = mesohaline, O = oligohaline, LD = low-dynamic intertidal, HD = high-dynamic intertidal, ST = subtidal

3.3.1.2 Macrofauna

In total, 24 different species were found, combined over all habitats and seasons (Supplementary material Tables 3.2-3.3, Figure 3.3 a-f). These comprise 12 surficial modifiers, 5 biodiffusors and 7 upward conveyors, of which 4 are both upward and downward conveyors. While the differences in species composition between the polyhaline and the other salinity zones were mainly caused by surficial modifiers, biodiffusors mostly determined the differences between the different habitat types (Figure 3.4).

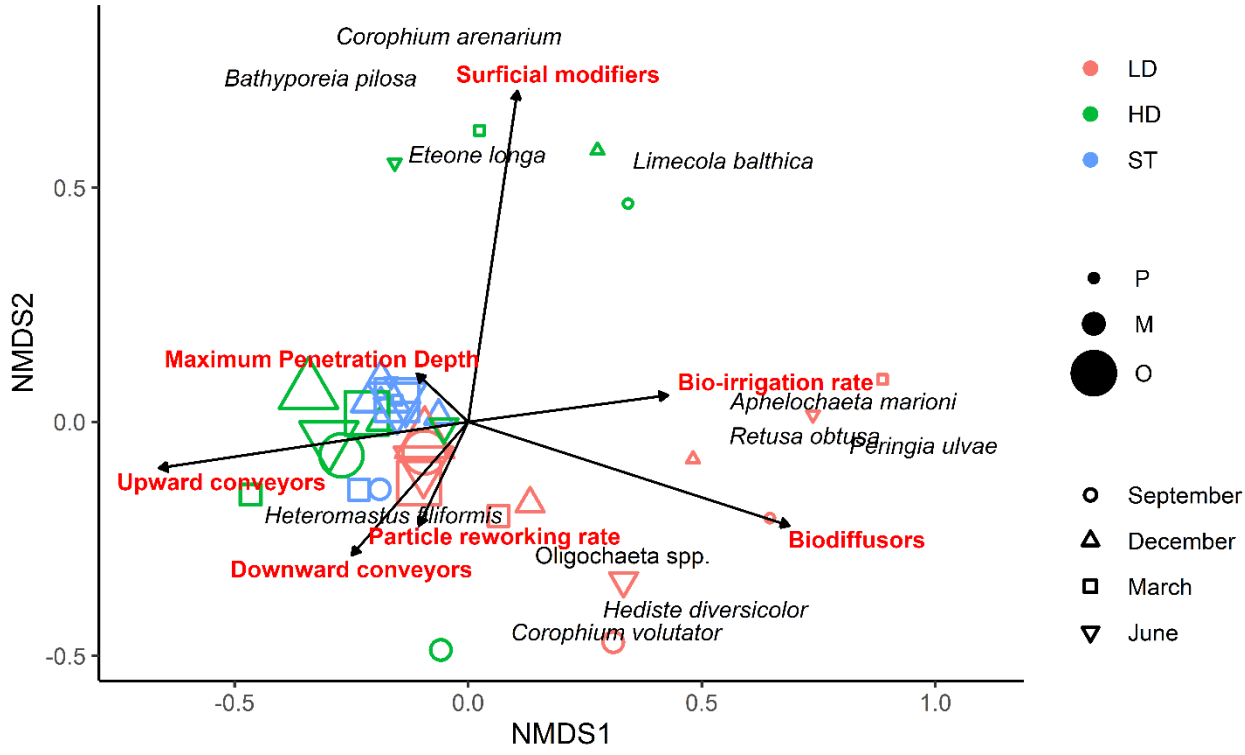


Figure 3.4: Non-metric multidimensional scaling based on macrofaunal densities, with vectors indicating directions of positive correlation (Pearson's product moment) with the four functional groups and bioturbation variables (particle reworking rate, bio-irrigation rate and maximum penetration depth). Only the centroids and species with correlations > 0.2 are shown to ensure readability of the plot. P = polyhaline, M = mesohaline, O = oligohaline, LD = low-dynamic intertidal, HD = high-dynamic intertidal, ST = subtidal.

The total macrofaunal densities in subtidal habitats, in which macrofauna was often absent, were always low (Supplementary material Tables 3.3-3.4, Figure 3.3 a-f). Densities fluctuated in the polyhaline low-dynamic intertidal habitat, with generally higher values in September and March and lower ones in December and June. A peak in March could also be observed in the oligohaline low-dynamic habitat. Densities in the polyhaline high-dynamic and mesohaline low-dynamic habitats gradually increased over the course of the survey. The densities of surficial modifiers peaked in June in the polyhaline high-dynamic and the mesohaline low-dynamic intertidal habitat, in March in the polyhaline low-dynamic intertidal habitat and in December in the oligohaline low-dynamic intertidal habitat. They were absent or occurred in low densities in the other habitats. Biodiffusors occurred in low densities in the subtidal and high-dynamic intertidal habitats, and showed fluctuating patterns in the low-dynamic intertidal habitats, with drops from September to December and from March to June, and increases from December to March. Upward and downward conveyors show generally similar patterns, since species were often classified in both groups (Supplementary material Table 3.2, Figure 3.3 a-f). Both groups were absent in the oligohaline zone and never reached densities above 900 ind m^{-2} in the other salinity zones. Densities peaked in March in the polyhaline low-dynamic and high-dynamic intertidal. Upward conveyors in the mesohaline zone reached peak densities in September in the intertidal habitats and in March in the subtidal habitat. Total biomass (AFDW) was lowest in the oligohaline zone and highest in the polyhaline zone. The low-dynamic intertidal habitats always displayed the highest total AFDW.

Bio-irrigation and estimated particle mixing rates manifested significant month*habitat interaction effects (Table 2.1; **Chapter 2**). In the polyhaline zone, bio-irrigation rates peaked in December in the high-dynamic intertidal habitat, in September in the low-

dynamic intertidal habitat and in March in the subtidal habitat. In the mesohaline zone, the overall highest values are recorded in March, while the low-dynamic intertidal habitat then drops to its lowest values in June. Patterns are difficult to distinguish in the oligohaline zone due to the exclusion of unreliable results (see Materials and methods), but a drop in all habitats from September to December can be observed, as well as a later increase in June. Particle reworking rates in the low-dynamic intertidal habitats peaked in December in the mesohaline and oligohaline zones, and in September in the polyhaline zone. In the latter salinity zone, particle reworking rates in the subtidal and high-dynamic intertidal habitats peaked in June (Supplementary material Table 3.4). Both processes have been more thoroughly investigated in **Chapter 2**.

3.3.1.3 Biogeochemistry

PERMANOVA revealed that a significant Month*Habitat*Salinity interaction effect for nitrite fluxes ($p = 0.007$; Table 3.1). Furthermore, differences in fluxes of nitrate, ammonia and oxygen between months depended on the habitat type (Month*Habitat interaction; $p<0.05$; Table 3.1), and also on the salinity zone for ammonium and oxygen flux (Salinity*Habitat effect). Finally, a significant Habitat x Salinity effect demonstrated that differences in oxygen fluxes between habitats also depended on the salinity zone. as well as for o ($p<0.05$; Table 3.1). No significant single and interactive effects of Habitat, Month, Salinity was found for phosphate fluxes.

Oxygen flux was always directed towards the sediment community (influx), and showed strongest absolute values in March or December in the high-dynamic intertidal habitats, and in September or June in the low-dynamic intertidal habitats (Figure 3.5; Supplementary material Table 3.5). The patterns of nitrate and nitrite fluxes were similar, with peaking effluxes in the low-dynamic intertidal habitats in June in the polyhaline zone and in March in the mesohaline zone. In the oligohaline low-dynamic intertidal habitat, the fluxes peaked as influxes in March. The subtidal and high-dynamic intertidal habitats were less variable, lacking strong peaks. Ammonium effluxes were overall highest in September and in the intertidal habitats. In December and March, influxes were recorded in most habitats. Phosphate fluxes were mostly effluxes in September and June, and mostly influxes in December and March. Exceptions were influxes in the polyhaline high-dynamic intertidal habitat in June, in the mesohaline subtidal habitat in September, and in the oligohaline low-dynamic intertidal habitat in June. In the subtidal habitats, all fluxes were usually less variable than in the intertidal habitats.

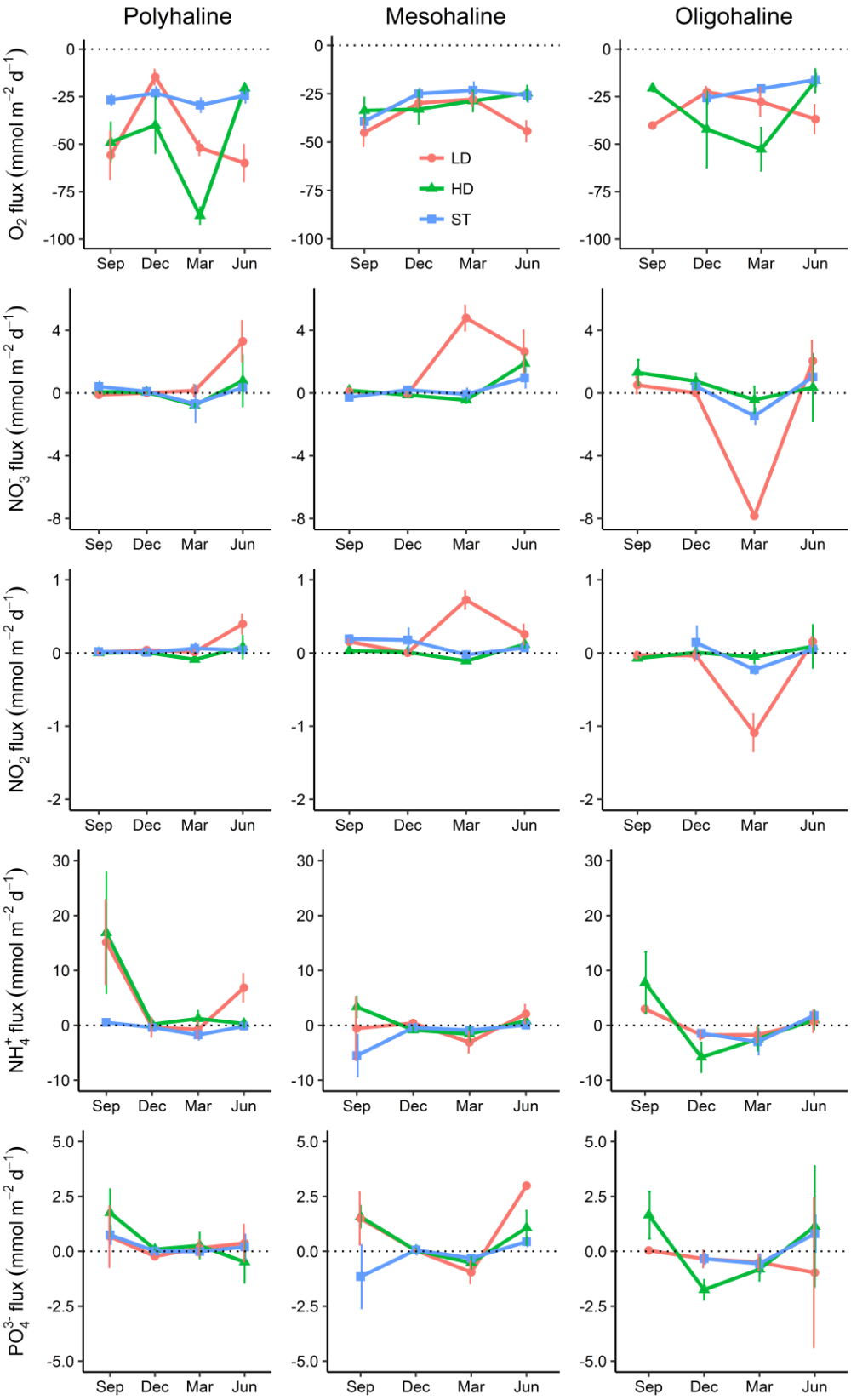


Figure 3.5: Overview of the fluxes of oxygen (O_2), nitrate (NO^{3-}), nitrite (NO^{2-}), ammonium (NH^{4+}) and phosphate (PO_4^{3-}), in mmol m^{-2} d^{-1}, per salinity zone, month and habitat (LD = low-dynamic intertidal, HD = high-dynamic intertidal, ST = subtidal). Error bars represent the deviation of standard errors from the

mean. A positive flux indicates an efflux from the sediment. Data points in each month were independent from each other.

Table 3.1: Spatio-temporal effects on functional variables (Fluxes of phosphate, nitrate, nitrite, ammonia and oxygen). (a) The results of PERMANOVA main tests. The last column shows the *p*-values of PERMDISP tests. Mo = Month, Sa = Salinity, Ha = Habitat. (b) Significant pair-wise differences between levels of the different factors, for which the main test was significant ($P < 0.05$). Sep = September, Dec = December, Mar = March, Jun = June, P = polyhaline, M = mesohaline, O = oligohaline, ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal.

(a) Variable	Effect	Pseudo-F	p	p (PERMDISP)
Phosphate	Mo	2.20	0.094	
	Sa	0.57	0.573	
	Ha	0.27	0.769	
	MoxSa	0.78	0.570	
	MoxHa	0.51	0.791	
	SaxHa	0.67	0.627	
	MoxSaxHa	0.62	0.762	
Nitrate	Mo	3.21	0.023	
	Sa	1.34	0.264	
	Ha	6.87	0.002	
	MoxSa	2.16	0.066	
	MoxHa	3.97	0.001	0.001
	SaxHa	0.96	0.433	
	MoxSaxHa	1.34	0.237	
Ammonia	Mo	4.97	0.002	
	Sa	5.04	0.007	
	Ha	1.74	0.184	
	MoxSa	2.84	0.013	0.01
	MoxHa	2.44	0.026	0.082

	SaxHa	0.75	0.581	
	MoxSaxHa	0.37	0.943	
Nitrite	Mo	1.25	0.299	
	Sa	1.89	0.152	
	Ha	8.64	<0.001	
	MoxSa	1.31	0.254	
	MoxHa	3.81	0.002	
	SaxHa	1.34	0.264	
	MoxSaxHa	2.85	0.007	0.004
Oxygen	Mo	4.52	0.005	
	Sa	9.36	<0.001	
	Ha	10.09	<0.001	
	MoxSa	5.14	<0.001	0.08
	MoxHa	8.02	<0.001	0.01
	SaxHa	3.65	0.009	0.01
	MoxSaxHa	1.6	0.134	

(b)	Month	Salinity	Habitat
Nitrate	HD: Sep-Mar, Dec-Mar		Mar: HD-LD, LD-ST
	LD: Sep-Mar, Sep- Jun, Dec-Mar,		Jun: LD-ST
	Dec-Jun		
Ammonia	M: Mar-Jun	Sep: M-P	Sep: HD-ST
	O: Dec-Jun, Sep-Dec	Dec: M-O, O-P	Jun: LD-ST
	P: Sep-Mar, Sep-Dec, Dec-Jun,		
	Mar-Jun		
	HD: Sep-Dec, Dec-Jun		

	LD: Sep-Mar, Mar-Jun, Dec-Jun ST: Dec-Jun		
Nitrite	MHD: Sep-Mar, Dec-Mar MLD: Sep-Dec, Sep-Mar, Dec-Mar MST: Sep-Mar PHD: Sep-Mar, Dec-Mar PLD: Sep-Jun, Dec-Jun, Mar-Jun	SepHD: M-O, M-P, O-P SepLD: M-O, M-P SepST: M-P MarLD: M-P	SepM: HD-LD, HD-ST DecP: HD-LD, LD-ST MarM: HD-LD, HD-ST, LD-ST MarP: HD-ST JunP: LD-ST
Oxygen	P: Mar-Jun, Dec- Mar M: Sep-Mar, Mar-Jun HD: Sep-Mar, Sep-Jun, Mar-Jun LD: Dec-Jun, Dec-Mar, Sep-Dec ST: Sep-Dec, Sep-Jun	Mar: M-P HD: M-P	Sep: LD-ST Mar: LD-ST, LD-HD, HD-ST Jun: HD-LD, LD-ST M: LD-ST P: HD-ST, LD-ST

3.3.2 Macrofauna and environmental variables explaining multivariate benthic flux variation

In DistLM analysis, the benthic fluxes were first modelled as a response to macrofaunal and environmental variables considering the entire dataset. The most parsimonious model selected total density, bio-irrigation and temperature as predictors (Table 3.2). When DistLM was applied to subsets of the dataset separated by seasons, macrofaunal descriptors dominated most of the year: bio-irrigation and maximum penetration depth in June, biomass of biodiffusors in September and density of downward conveyors in December, while the model applied in March selected mostly environmental factors: chlorophyll a concentrations and mud content.(Table 3.2). In the models based on habitat-defined subsets, environmental variables turned out to explain a greater portion of variability in benthic fluxes. Only the model for low-dynamic intertidal habitats

retained both macrofaunal (Total biomass and biomass of biodiffusors) and environmental predictors (temperature). In high-dynamic intertidal habitats, temperature and median grain size were selected, while temperature was the sole predictor for ecosystem functioning in subtidal habitats (Table 3.2).

When benthic fluxes were modelled accounting for the entire dataset, the low R^2 values call for caution in the interpretation of the results. In the model for the low-dynamic intertidal, the proportion of the constrained variance was higher than that of the unconstrained variance, with a resulting R^2 value of 0.6; the constrained variance for the March models approximated highest portions of the total variance with R^2 values of 0.86 (Table 3.2).

Table 3.2: Model parameters of all DistLM models. Var. Part = variation partitioning, showing the R^2 values for the variance explained by environmental (E) and macrofaunal (M) variables; ST = subtidal; HD = high-dynamic intertidal; LD = low-dynamic intertidal; HD = high-dynamic intertidal.

Model	Predictors	Proportion explained	Var. Part.	R^2
All	Total density	0.13	E: 0.05	0.23
	Bio-irrigation	0.05	M: 0.18	
	Temperature	0.05		
September	BiodiffusorAFDW	0.3	M: 0.3	0.3
December	Downward conveyor	0.35	M: 0.4	0.4
	Total density	0.05		
March	ChlorophyII a	0.48	E: 0.79	0.86
	Mud content	0.31	M: 0.07	
	Downward conveyor	0.07		
June	Mud content	0.15	E: 0.15	0.37
	Maximum penetration depth	0.15	M: 0.22	
	Bio-irrigation	0.07		
ST	Temperature	0.22	E: 0.22	0.22

HD	Temperature	0.21	E:0.38	0.38
	Median grain size	0.17		
LD	Temperature	0.35	E: 0.35	0.6
	Total AFDW	0.12	M: 0.25	
	Biodiffusor AFDW	0.13		

3.4 Discussion

The estuary of the Scheldt has been intensively studied and its macrobenthic communities are well characterised. Their spatial, and to a lesser extent also temporal occurrences have been shown to be mostly controlled by environmental conditions (Ysebaert and Herman, 2002), with salinity being the dominant factor in explaining macrobenthos distribution and diversity (Ysebaert et al., 1998). However, this is not necessarily the case for the benthic fluxes. Research in other estuarine systems has shown that sediment biogeochemistry changes along the salinity gradient (Bordalo et al., 2016; Tomaso and Najjar, 2015), as well as in function of the sediment properties of the habitats (Eyre et al., 2013).

3.4.1 Spatio-temporal variability in sediment biogeochemistry

Our values for the oxygen or nutrient fluxes in the Scheldt estuary lie within the range found in other European estuaries with a similarly heavy human impact, such as those of the Seine, Thames, Tagus and Douro (Bally et al., 2004; Cabrita and Brotas, 2000; Magalhães et al., 2002; Trimmer et al., 2000). The high spatial and temporal variation in biogeochemical fluxes illustrates the complexity of the environmental influence on different components of the Scheldt estuary ecosystem. Overall, we could make a distinction between the warmer months of the year (September, June) and the colder months (December, March). For example, in many habitats, oxygen fluxes were higher and nutrients effluxes rather than influxes into the sediment could be distinguished

during the warmer months. The highest flux rates and oxygen consumption were generally found in September or June, when temperatures were highest, corresponding to findings in other marine and estuarine systems (Cabrita and Brotas, 2000; Eriksson et al., 2003; Roberts and Doty, 2015). Some habitats or salinity zones, however, deviated from this general pattern. The subtidal habitats in particular tended to exhibit less variability in biogeochemical flux rates, which can probably be attributed to the extremely low densities or even absence of macrobenthos and their reworking and ventilating behaviour.

3.4.2 Macrofaunal and environmental contributions to benthic biogeochemistry

The spatial and temporal variability in biogeochemical fluxes is caused by different combinations of macrofaunal and environmental factors. Elevated oxygen fluxes or strong nutrient fluxes in the warmer months can be attributed to the higher temperatures, stimulating (microbial) metabolic rates (Lopez-Urrutia et al., 2006). However, in the high-dynamic intertidal habitats in the poly- and oligohaline zones, an opposite pattern was observed for oxygen fluxes, with higher values in March. The high oxygen fluxes in the oligohaline high-dynamic intertidal in that month could be explained by relatively higher levels of organic matter in the sediment as compared to the other months, possibly due to import of terrestrial material after winter storms. Moreover, densities of known bio-irrigating species were high during December and March in both the polyhaline (*C. arenarium* and *B. pilosa*) and oligohaline zone (*B. pilosa*). The bio-irrigating activity of the macrofauna showed – at least in the polyhaline zone – similar patterns to those of oxygen fluxes, suggesting that increases in oxygen consumption are at least partly caused by increases in macrofaunal activity. Furthermore, bio-irrigation rates correlated positively with oxygen fluxes (Figure 3.5), indicating the importance of burrow ventilation as a

means of injecting oxygen into the sediment, stimulating oxygen-consuming processes (Glud, 2008).

Especially in the low-dynamic intertidal habitat of the oligohaline zone, the nitrate and nitrite fluxes seemed to correspond well to the densities of biodiffusors (Figure 3.5; Supplementary material Figure 3.3 c.). Even though data are missing for bio-irrigation in March in that salinity zone, the nutrient fluxes suggest that the higher densities of biodiffusors increased coupled nitrification-denitrification indicated by higher nitrite and nitrate effluxes (Figure 3.5), probably through bio-irrigation (Cornwell et al., 1999; Pelegri et al., 1994). All fluxes showed less seasonal variation in the subtidal habitats than in the intertidal ones (Figure 3.5), most likely due to the low abundances of macrofauna, and therefore lower fauna-mediated fluxes (Wrede et al., 2017). Summer effluxes of ammonium were most likely caused by increased decomposition of organic matter, coincident with higher temperatures and an increased microbial and macrofaunal activity (Gao et al., 2014; Noe et al., 2013).

Biodiversity can play a crucial role in determining rates of key ecosystem processes (Cardinale et al., 2012), a relationship that has been illustrated in marine ecosystems as well (Belley and Snelgrove, 2017; Godbold and Solan, 2009; Waldbusser et al., 2004). Many of these studies have equated diversity to taxonomic diversity, but taxonomy says little about the roles that species play in ecosystem functioning. A more direct link should become apparent when assigning species to functional groups or considering community-wide processes such as particle mixing and bio-irrigation. The selection of bio-irrigation rates in the general model demonstrates the importance of ecosystem process to benthic biogeochemistry. Nevertheless, the low fit of the model and the low proportion of constrained variance point out that other factors are at play as well. Meiofauna was not assessed in this study, but can play an important role in sediment

processing and biogeochemical cycling by bioturbating or feeding in the sediment (Schratzberger and Ingels, 2018). Densities and diversity of microbiota, being directly responsible for biogeochemical fluxes, were also not measured. These organisms are also known to be heterogeneously distributed in estuaries, following spatial and temporal gradients (Foshtomi et al., 2015; Hamels et al., 1998). In general models performed significantly better when based on season- or habitat-defined subsets of the total dataset, suggesting that different factors may become more prominent depending on the season or habitat in the estuary.

Remarkably, we observed a change from a mostly environmental to a purely macrofaunal contribution to benthic fluxes in the first half compared to the last half of the year, when the biomass of biodiffusors (September) or density of downward conveyors (December) had their greatest impact on the variation of benthic biogeochemistry. Temperature is probably the main driver for these changes, which is best illustrated in March, when temperatures and particle reworking activity were low and environmental contribution to the biogeochemistry at a maximum. At that time, fluxes appear to be regulated much more by the physico-chemical characteristics of the sediment related to its organic loading, i.e. chlorophyll *a,* and are dominated by microbial processes. As it gets warmer later in the year, the macrofaunal contributions gradually take over. In June, particle reworking (selected here as the maximum penetration depth) and bio-irrigation had a higher explanatory contribution than environmental properties f, indicating a rising contribution of the macrofauna to benthic biogeochemistry. The macrofaunal contribution eventually becomes dominant and this remains so till December, even when temperatures drop again.

The explanatory contribution of temperature to variation in benthic fluxes was consistent between the three main habitats (Table 3.2). In the subtidal habitat, macrofauna had

extremely low densities or was even absent, and sediment biogeochemistry was only governed by temperature-controlled microbial processes. In the high-dynamic intertidal habitat, faunal densities were similarly low in all except the polyhaline salinity zone, which may explain the effect of salinity on the ammonium and oxygen fluxes, that in part reflects the salinity-dependent occurrence of the fauna. Similarly, temperature contributed most to the variability in the biogeochemistry of the macrofauna-rich low-dynamic intertidal habitat, but the total biomass and biomass of biodiffusors explained an additional 25 % of the variation similar to what is known for many other coastal ecosystems (Cowan et al., 1996; Fulweiler et al., 2010; Hopkinson et al., 2001). It is noteworthy that patterns in biodiffusor densities correspond to those of the bio-irrigation rates in the low-dynamic intertidal habitat, as some biodiffusors, most notably the polychaete *H. diversicolor*, are indeed known as important bio-irrigators (Duport et al., 2006; Renz and Forster, 2014; Van Regteren et al., 2017).

The baseline knowledge attained from the seasonal survey in the Scheldt estuary can be further applied to partition the variation caused by future climate changes and other anthrophonic disturbances, such as dredging and dumping, that change benthic habitats and benthos animals. Changes in ambient temperature will alter individual growth rates and other physiological functions of benthic organisms in estuarine environments (Beukema et al., 1993, 1998). The possibility of milder winters and warmer summers in the future implies that the overall biomass of estuarine benthic assemblages may be expected to increase (Widdows et al., 2002). Thus, the bioturbation activity might become higher, further affecting ecosystem functioning. Additionally, the regulation of food availability in benthic biomass and activities as revealed in the survey data in the Scheldt estuary is aligned with other available survey data from the estuarine systems worldwide (Herman et al., 1999a). The relationship between food availability and macrobenthos

established in the survey data represents a baseline status, which provides the potential to partition the variation caused by the long-term climate change and associated sea-level rise. However, challenges remain to evaluate the effects of climate change on intertidal macrobenthos since it has not been possible to determine the degree to which any loss of invertebrate biomass resulting from other environmental alterations (e.g. anthropogenic influence, ocean acidification) may be compensated by these factors.

3.5 Conclusion

This study showed that biogeochemical fluxes and functional properties of the macrobenthic communities vary across different habitats and seasons in the estuary of the Scheldt. Furthermore, this study showed that the environmental variables and macrofauna properties we measured can explain a significant part of the variability in biogeochemical fluxes at our study sites. However, the relative contributions of environmental and macrofaunal properties to the variability in benthic biogeochemistry were different in each habitat and season. We confirmed our first hypothesis that processes linked to the biota were lowest and overruled by environmental steering in the coldest period of the year, while processes related the burrowing behaviour of macrofauna, or densities and biomasses of specific sediment reworking functional groups predominantly determined biogeochemical variability from June onwards. Secondly, the relative macrofaunal contribution to the biogeochemistry was highest in low-dynamic intertidal habitats that are densely inhabited by biodiffusing and bio-irrigating fauna. In conclusion, this study illustrates that habitat changes and loss of functional groups of macrofauna will affect ecosystem functions such as benthic-pelagic flux rates that underpin the delivery of ecosystem services.

Acknowledgements

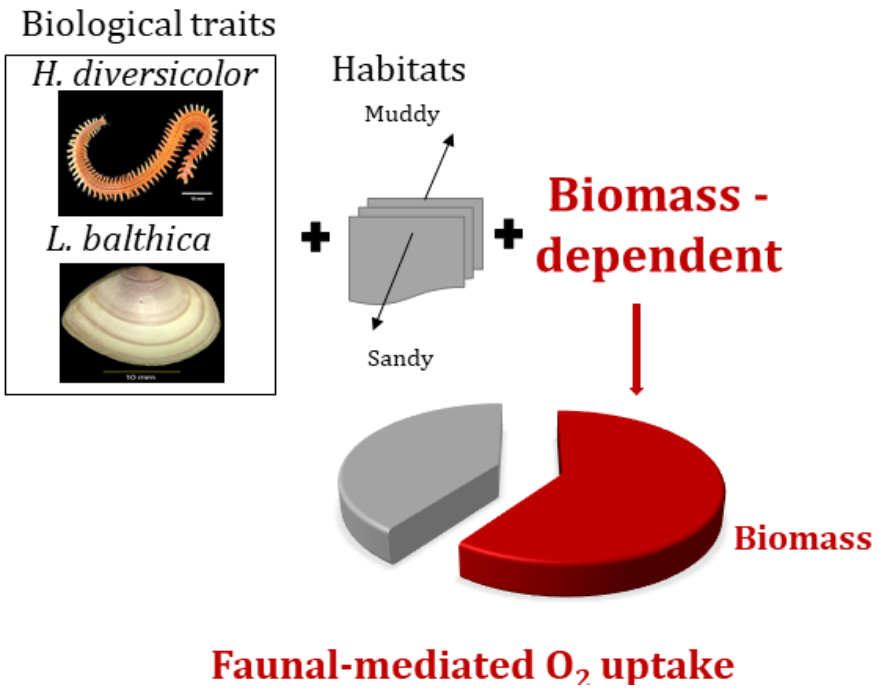
Xiaoyu Fang received a scholarship from the MARES doctoral programme (2012-1720/001-001-EMJD) and Sebastiaan Mestdagh received a scholarship from the Special Research Fund of Ghent University (BOF.DOC.2014.0081.01). Part of the results presented in this publication were obtained using infrastructure funded by EMBRC Belgium – FWO project GOH3817N. We would like to express our gratitude to the crew of *RV Simon Stevin* and to laboratory technicians Dirk Van Gansbeke, Bart Beuselinck, Jan Sinke and Peter Van Breugel for analyses of samples. Furthermore, special thanks to Guy Desmet, Mohammed Alsebai, Katja Guilini, Christophe Verwimp, Annick Van Kenhove, Niels Viaene, Elise Toussaint, Emil De Borger, Ninon Mavraki, Ee Zin Ong and Thomas Bauwens for assistance at least at one point during one of the sampling campaigns or laboratory experiment.

Chapter 4 Benthos contribution to ecosystem functioning depends on habitat, species identity and biomass

Abstract

Estuarine ecosystem functioning is strongly influenced by the bioturbation (i.e. particle mixing and bio-irrigation) of macrobenthos. The impact of two bioturbating benthic invertebrates, *Limecola balthica* and *Hediste diversicolor*, on sediment community oxygen uptake in the Scheldt estuary was examined. Microcosms containing defaunated sediment with artificially composed faunal densities were used to test how species identity, habitat, species body size and population density influence O_2 uptake in different habitats (muddy and sandy sediments). Both *L. balthica* and *H. diversicolor* facilitated O_2 fluxes between the sediment and the overlaying water, and a major portion of the variance in sediment aerobic metabolism and bio-irrigation could be explained by the per capita body size and density, or by the total biomass of the inhabiting bioturbators. *H. diversicolor* showed a more pronounced relationship between biomass/density and faunal-mediated O_2 consumption than *L. balthica*. Both *H. diversicolor* and *L. balthica* irrigate the sediment, the former by ventilating their burrows the latter by siphoning water. A significant linear relationship was found between *H. diversicolor* bio-irrigation and the corresponding faunal-mediated O_2 uptake, whilst irrigation from *L. balthica* related linearly to the faunal-mediated O_2 consumption only in the sandy habitat. ANCOVA suggested significant habitat effects in bio-irrigation patterns, which might be attributed to different physical constraints (e.g. O_2 availability) in sandy and muddy sediments. In summary, we demonstrated that the faunal-mediated O_2 consumption by *H. diversicolor* and *L. balthica* is density- and biomass- dependent, but that the mediating

mechanisms of benthic bioturbation vary according to species-specific biological traits and sediment physico-chemical properties.



Graphical abstract of Chapter 4

This Chapter is in preparation as:

Benthos contribution to ecosystem functioning depends on habitat and species identity and biomass

Xiaoyu Fang, Karline Soetaert, Antony Knights, Tom Moens, Carl Van Colen

4.1 Introduction

Estuaries and coastal marine ecosystems are considered among the most productive biomes of the world and serve as important life-support systems (Costanza et al., 1997; Day et al., 2013). The functioning in these ecosystems is strongly influenced by the activities of different taxa living in the seabed (Levinton, 2011; Meysman et al., 2006a; Rhoads, 1974). Particularly, ecosystem services such as gas regulation (e.g. O_2 uptake), the bioremediation of waste, and the provision of biologically-mediated habitat depend on the activities of benthic animals (de Bello et al., 2010; De Valença et al., 2017; Kristensen et al., 2012; Wilkinson et al., 2009). Macrofauna are an important group of benthic animals that affect benthic metabolism due to their high biomass and behavioural activities (Lohrer et al., 2004b). A key process is the modification of sediments through bioturbation that encompasses two main processes: particle reworking (Maire et al., 2008; Solan and Wigham, 2005) and bio-irrigation (Kristensen and Kostka, 2005; Meysman et al., 2006c). Particle reworking results from a series of sediment mixing processes that transport particles and associated living and non-living substances through faunal feeding, defaecation and burrowing activities, whilst bio-irrigation is the ventilation of burrows with seawater by the fauna providing dissolved substances for respiration and particles for feeding purposes and removing reduced substances from the sediment by re-oxidation (Kristensen et al., 2012; Riisgård and Larsen, 2005).

Macrobenthos influence all O_2 dependent biogeochemical processes within sediments, either directly through aerobic respiration, or indirectly through particle mixing and burrow ventilation that redistribute reduced compounds and aerate the sediment, thus providing microbial habitats for intensified biogeochemistry (Bonaglia et al., 2014; Sundbäck et al., 2004; Van Colen et al., 2012). The sediment community O_2 consumption (SCOC) is the most widely used measure of benthic metabolism (Thamdrup and Canfield,

2011) as it represents a proxy for total benthic carbon mineralization rate (Canfield et al., 1993), integrating the reoxidation of anaerobic substances plus aerobic respiration of benthic organisms. Depending on the environmental context (e.g. sediment permeability, organic loading, temperature) and species traits (feeding mode, living position, population density, body size), the stimulation of O_2 consumption via macrofaunal bioturbation can outweigh their direct O_2 consumption by respiration (Glud et al., 2003, 2000).

Activity of organisms depends on their metabolism (Brown et al., 2004; Savage et al., 2004) which varies with body size (Gaston and Blackburn, 2007; Marquet, 2005). Consequently, ecological scaling laws based on body size allometry have been applied to predict the impact of macrofauna on sediment parameters such as respiration and bioturbation (Cozzoli et al. 2018). Allometric laws based on body size and abundance can be used to upscale from individual to population-level effects (De Roos et al., 2003) and are thus of practical value for e.g. size-based assessments of ecosystem functioning (e.g. Blanchard et al. 2009). The allometric scaling of species effects on sediment metabolism can be assumed via the respiration, feeding and moving rates. An organism's metabolic rate is expected to scale with individual size with an allometric exponent of ~0.75 (Kleiber, 2014; West et al., 1997). However, large environmental (Barneche et al., 2014; Yvon-Durocher et al., 2012), taxonomic (Barneche and Allen, 2015; Isaac et al., 2011) and phenotypic (Clark et al., 2016) variability may influence the actual values of the scaling exponents. In addition to body size, population density, sediment reworking mode, and the abiotic habitat properties determine the role of macrofauna on sediment metabolism. This is because macrofaunal mediating effects are expected to vary strongly between diffusion-dominated and advection-dominated systems (Boulton et al., 2002). For example, the macrofauna contribution to aerobic sediment metabolism is limited in

permeable, well-oxygenated sediments as compared to cohesive organically enriched sediments (Mermillod-Blondin and Rosenberg, 2006). In addition, species activities respond to abiotic conditions (Liancourt et al., 2005; Norkko et al., 2006), such as temperature, hydrodynamic regimes, resource availability and quality (Fang et al., 2019; Mrowicki and O'Connor, 2015; O'Connor et al., 2015; Törnroos et al., 2015). Metabolic rates of organisms are also strongly dependent on the environmental temperature according to a positive Boltzmann-Arrhenius relationship (Brown et al. 2004). The contribution of macrofauna to ecosystem metabolism may thus fundamentally change over time and across space (Godbold et al., 2011; Levinton and Kelaher, 2004; Needham et al., 2010).

In this study we quantify the biomass-dependent influence of two macrofauna species (*H. diversicolor* and *L. balthica*) on the sediment metabolism of an estuarine mudflat and sandflat habitat. The gallery burrowing omnivorous polychaete *H. diversicolor* and the facultative deposit feeding tellinid bivalve *L. balthica* have different particle mixing and irrigation modes and represent two common species in temperate estuaries where they typically constitute a significant part of the macrofauna biomass (e.g. Ysebaert et al., 2003). Laboratory monocultures with various abundances and sizes of individuals were used to quantify population irrigation rates and the mediating effect of both species on sediment O_2 uptake in both habitats. We hypothesized that (1) allometric principles of metabolism scaling with population density can be applied to quantify the faunal effects on sediment O_2 uptake; and (2) biological traits (e.g. bio-irrigation) and sediment physico-chemical conditions significantly affect faunal-mediated O_2 consumption.

4.2 Materials and Methods

4.2.1 Study sites and target organisms

Sediments and organisms were collected from Paulina intertidal flat, in the polyhaline reach (average salinity 24-32) of the Scheldt estuary. There are a variety of habitats on this tidal flat, varying from muddy to sands almost devoid of silt (Gallucci et al., 2005). A sandy location (51°21' 00.2" N, 3° 43' 54.9" E) and a muddy location (51° 20' 57.1" N, 3° 43' 35.4" E) were chosen (Figure 4.1). Sediment composition, organic matter content, permeability and O_2 penetration depth vary between both habitats. An overview of sediment characteristics can be found in Table 4.1 Oxygen penetration depths were derived from the vertical oxygen profiles measured in seasonal survey on day 4 and 5 of the experiment using Unisense oxygen microsensors (type OX100) in vertical increments of 250 µm (for timeline of experiment scheme refer to Figure 3.2). Permeability was calculated based on Eggleston and Rojstaczer (1998): KH = 1.1019×103 m^{-2} s * d102 * ν, where KH is the permeability (in m^2), d_{10} is the first decile of the grain size distribution (in m), and ν is kinematic viscosity (in m^2 s^{-1}, calculated from water temperature and salinity in baseline survey). The methodology for sediment composition and organic matter quantification is presented in **Chapter 2**.

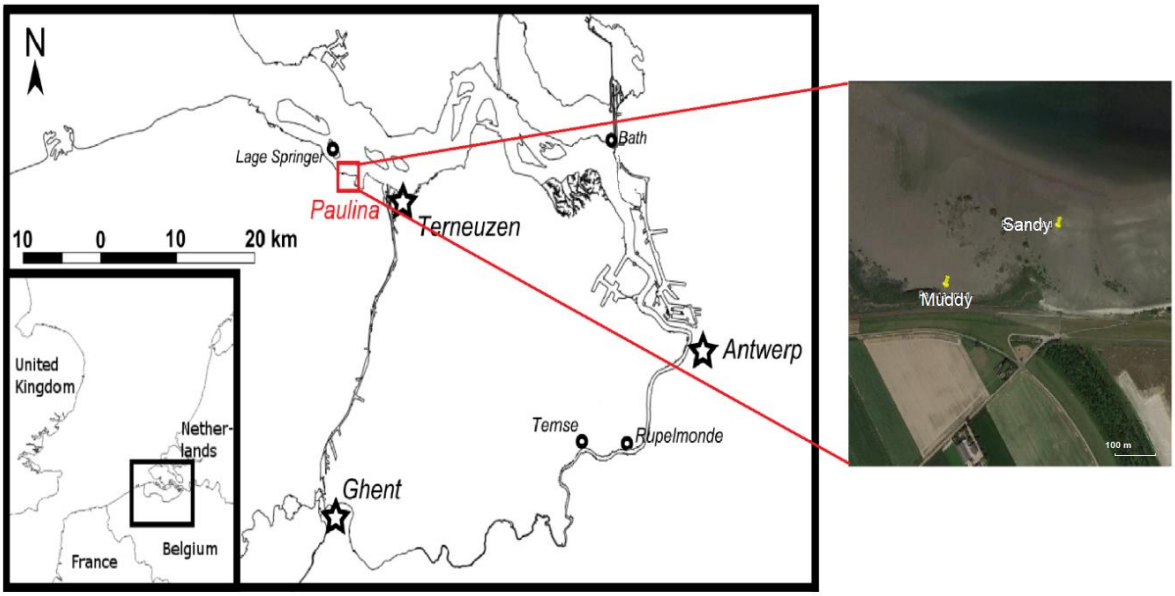


Figure 4.1: Map of the Scheldt estuary, with two sampling locations (sandy and muddy sediment) indicated on Paulina tidal flat.

Table 4.1: Abiotic sediment characteristics for the two sampling locations: median grain size (in µm), mud content (in % < 63 µm), total organic carbon (in %), permeability (in m^2) and oxygen penetration depth (in mm). Data were derived from the baseline survey in the Scheldt estuary 2015-2016 (**Chapter 2-3**) and the seasonal sampling campaigns were performed on 1-3 September 2015, 8-10 December 2015, 1-3 March 2016, and 21-23 June 2016. All values are means ± standard errors.

Time	Habitat	Median grain size (µm)	Mud content (%)	TOC (%)	Permeability (m^2)	Oxygen penetration depth (mm)
Sep. 2015	Sandy	221 ± 0.06	3 ± 0.4	0.21 ± 0.05	2.04E-11±4.62E-13	4.38±1.38
	Muddy	47 ± 0.5	64 ± 0.6	1.22 ± 0.05	6.60E-14±4.00E-15	3.69±0.55
Dec. 2015	Sandy	227 ± 2	3 ± 0.5	0.21 ± 0.02	2.78E-11±1.01E-12	9.25±0.75
	Muddy	46 ± 0.7	65 ± 0.4	1.24 ± 0.07	8.67E-14±6.67E-15	4.44±0.26
Mar. 2016	Sandy	227 ± 2	5 ± 0.6	0.20 ± 0.06	3.14E-11±2.32E-12	11.42±1.58
	Muddy	47 ± 0.3	63 ± 0.4	1.22 ± 0.02	8.68E-14±4.39E-15	4.06±0.72
Jun. 2016	Sandy	228 ± 0.5	0.3 ± 0.3	0.09 ± 0.03	2.47E-10±1.67E-13	5.75±2.25
	Muddy	44 ± 0.3	67 ± 0.3	1.11 ± 0.03	4.73E-14±1.60E-15	3.67±0.51

L. balthica and *H. diversicolor* are ubiquitous in the polyhaline reach of the Scheldt estuary, occupying both muddy and sandy sediments. *H. diversicolor* lives in a mucus-lined gallery of burrows extending 6 to 12 cm into the sediment (Davey, 1994). By muscular movements of the body, this species creates a more or less continuous current of water carrying O_2 and food particles into the burrow (Pelegrí and Blackburn, 1995; Riisgård and Riisgard, 1991). The feeding modes of *H. diversicolor* range from surface deposit to suspension feeding, and they may also actively scavenge and prey on small invertebrates (conspecifics included) (Aberson et al., 2011; Riisgård and Riisgard, 1991). It reworks and irrigates the sediments thereby affecting chemical fluxes, microbial processes, and sediment erosion/elevation (de Deckere et al., 2000; Kristensen and Mikkelsen, 2003). In the Scheldt estuary, it is often found high in the intertidal zone in the polyhaline zone, where its averaged density is 466 ind m^{-2}, or 1.7 gAFDW m^{-2}, with maxima up to 3928 ind m^{-2} (13.7 gAFDW m^{-2}) (Ysebaert et al., 1998). *L. balthica* is a facultative surface-deposit feeder with separate inhalant and excurrent siphons, foraging on microphytobenthos, bacteria and labile organic matter present in the surface sediment (Kamermans, 1994). They intermittently increase the pressure in the sediment during feeding, as they take up surface particles and overlying water via the inhalant siphon while the excurrent siphon remains several cm deep in the sediment (Volkenborn et al., 2012). The subsurface water injection can be at a depth of 2-10cm in the sediment (Woodin et al., 1998). *L. balthica* switches between suspension feeding and surface-deposit feeding in response to changes in food conditions, but also depending on abiotic factors like tidal phase, current velocity and both quantity and nutritional quality of suspended particles (De Goeij and Honkoop, 2002; Lin and Hines, 1994). Individual body size and community composition also impact their feeding mode (Rossi and Middelburg, 2011). In the polyhaline zone of the Scheldt estuary, the mean density of *L. balthica* is 855 ind m^{-2} and the maximum density is 5217

ind m^{-2}, with a corresponding mean and maximum biomass of 2.9 and 15.9 gAFDW m^{-2} (Ysebaert et al., 1998).

4.2.2 Experimental design

To provide mechanistic insight into the relationship between aerobic sediment metabolism and benthic population density, we experimentally tested single-species treatments spanning a broad range of natural body size and abundance. Based on the natural occurrence of the species in the Scheldt estuary, a factorial experimental design consisting of the two invertebrate species (*L. balthica* and *H. diversicolor*) in a monoculture crossed with two polyhaline habitats (muddy, sandy) was adopted.

Experiments were performed with species added to defaunated sediments. Sediments were collected in May 2017 using plexiglas cores (inner diameter 9 cm), sectioned into 0-1, 1-2, 2-4, 4-6 cm sediment fractions and wet sieved (1 mm mesh size) to remove macrofauna and large particles (Figure 4.2; Supplementary material Figure 4.1). Each sediment section was incubated at 15°C in aerated seawater collected from the study site and allowed to deposit prior to reconstruction of the sediment matrix. A rectangular container (19.4×29.4×15 cm) was consecutively filled with the sieved sediment added in corresponding order to reconstruct the vertical gradients of sediment properties, by overlaying the 2-4 cm layer to the deepest layer (4-6 cm), followed by the subsequent overlays of 1-2 cm and 0-1 cm sediment layers. In total, 4 rectangular containers filled with reconstructed and defaunated sediments was incubated at 15°C for at least one month before the experiments started (Figure 4.2).

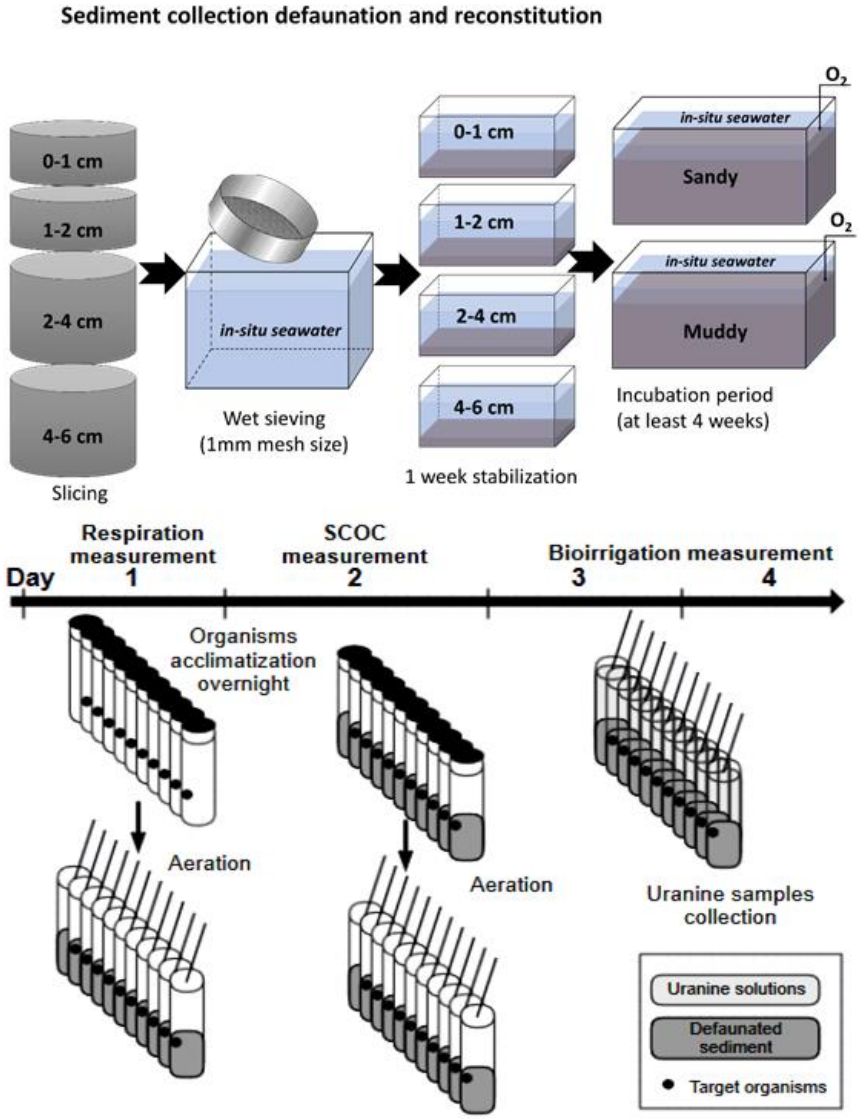


Figure 4.2: The timeline of sediment reconstitution and experiment scheme. The set-up contains one control core and ten microcosms with different density levels. Cores are shown as during O_2 flux measurement (capped) and bio-irrigation measurement (uncapped with continuous oxygen supply).

Organisms were collected with natural sediments on 15th June 2017 and left to acclimatize in the natural sediments at 15°C in containers with aerated seawater (salinity 20) collected from both sampling locations until the start of the experiments. Four separate experimental series were performed in June during which the total O_2 consumption and bio-irrigation were measured in plexiglass microcosms (height 12.2 cm, inner diameter 3.6 cm) following the same experimental routine (Figure 4.2;

Supplementary material Figure 4.1). The treatments included 10 density levels within the natural range for the two species and in the two habitats, giving a total of 4 experiment schemes and 40 microcosms. In addition, one additional microcosm that did not contain macrofauna was included in each experiment scheme (i.e. each experimental run) to distinguish the contribution of microbes and meiofauna activities in the defaunated sediment. A summary of the densities used for the target organisms and the habitats can be found in Table 4.2. Similarly-sized individuals of *H. diversicolor* ranging 1.7-45.7 mg AFDW ind^{-1} in the sandy habitat and 2.82-48.09 mg AFDW ind^{-1} in the muddy habitat (Table 4.2a) were selected for the treatments. For the *L. balthica* treatment, the body sizes of the added animals ranged from 3.4 to 31.41 mg AFDW ind^{-1} and from 1.98 to 22.4 mg AFDW ind^{-1} in muddy and sandy habitats respectively (Table 4.2b).

Table 4.2: Table of treatments. Several combinations of individual abundances and individual body sizes of target organisms (a) *H. diversicolor* (b) *L. balthica* were tested according to their natural range of density levels in polyhaline zone high-dynamic and low-dynamic intertidal habitats. Abundance in $Ind.m^{-2}$, Biomass in g $AFDW.m^{-2}$, Individual body size in mg AFDW ind^{-1}.

(a) *H. diversicolor*	Sandy			Muddy		
Treatment	Abundance	Biomass	Body size	Abundance	Biomass	Body size
1	0	0	0	0	0	0
2	943	7.6	8.06	943	2.66	2.82
3	943	28.51	30.22	943	14.59	15.47
4	943	43.11	45.7	943	45.37	48.09
5	1887	17.27	9.15	1887	27.82	14.74
6	1887	3.21	1.7	1887	21.74	11.52

7	2830	105.56	37.3	2830	71.29	25.19
8	3774	114.21	30.26	2830	46.66	16.49
9	4717	61.47	13.03	3774	99.26	26.3
10	4717	42.42	8.99	3774	43.09	11.42
11	5660	127.89	22.59	5660	32.68	5.77

(b) *L. balthica*

	Sandy			Muddy		
Treatment	Abundance	Biomass	Body size	Abundance	Biomass	Body size
1	0	0	0	0	0	0
2	934	7.08	7.58	943	4.81	5.1
3	934	24.15	25.86	943	16.04	17
4	934	29.34	31.41	943	21.13	22.4
5	1868	48.87	26.16	1887	32.74	17.35
6	2802	9.53	3.4	2830	11.79	4.17
7	2802	49.53	17.68	2830	18.87	6.67
8	2802	67.08	23.94	2830	51.42	18.17
9	4670	27.83	5.96	4717	17.64	3.74
10	4670	67.45	14.44	4717	40.28	8.54
11	5604	36.51	6.51	5660	11.23	1.98

The O_2 consumption in the water column was measured using robust O_2 optodes (Pyroscience robust O_2 probe OXROB10) connected to a FireSting O_2 meter (FSO_2-4, 4 O_2 channels). After pre-calibration using a two-point calibration with seawater at 0% and 100% O_2 saturation and considering the seawater salinity, each O_2 optode was inserted through a cap with an inserted rubber stopper keeping the sediment cores airtight. Microcosms were put on a platform shaker which oscillated horizontally at the speed 55 RPM, to assure homogenous mixing of O_2 in the water column. The slope of the temporal changes in dissolved O_2 concentrations was used to calculate the O_2 consumption, considering both the sediment surface and the volume of the overlying water.

Total O_2 uptake measured in microcosms filled with *in situ* seawater on day 1 was quantified as faunal respiration by subtracting the O_2 uptake only from seawater (i.e. control core without organisms) (Figure 4.2; Supplementary material Figure 4.2). To exclude the organisms biovolume from the water volume, a calibration relationship was established between Biomass (AFDW, in g) and biovolume (in ml) for populations from both habitats. Biovolume was determined from volume displaced when individuals were immersed in a known volume of water in a graduated cylinder (Persoone, 1971). The empirical functions ($p<0.05$) were: *H. diversicolor*, Biovolume=9.07AFDW+0.03 (R^2=0.94; Sandy habitat), Biovolume=8.63AFDW+0.06 (R^2=0.88; Muddy habitat); *L. balthica*, Biovolume=6.88AFDW+0.06 (R^2=0.91; Sandy habitat), Biovolume=7.34AFDW+0.01 (R^2=0.70; Muddy habitat) (Supplementary material Table 4.1).

Sediments were collected till approximately 6 cm deep from the reconstructed sediments and filled with 6 cm height of *in situ* seawater to construct the microcosms for measuring sediment community oxygen consumption (SCOC). The microcosms were aerated, and the organisms (of which the biovolume and individual oxygen uptake was estimated on day 1-see above) were carefully put on top of the sediment and left to acclimatize and

burrow overnight with aeration. An additional microcosm was established by solely adding sediments (~6 cm) and *in situ* seawater (~6cm), to estimate SCOC without macrofauna. On day 2, the O_2 uptake by the sediment community was measured with the same experimental set-up (i.e. 1 abiotic control and 10 species treatments) as day 1 and lasted until the O_2 concentration dropped to 60% of the initial concentration. Faunal-mediated O_2 uptake in the microcosms with sediments was calculated by subtracting the O_2 uptake measured in the abiotic control (defaunated sediments without animals). This faunal-mediated O_2 uptake includes both macrofaunal respiration and stimulation of respiration by bacteria and meiofauna.

Following the SCOC measurements, sediments were aerated, and the organisms were allowed to acclimatise overnight. On the third day, seawater was replaced with a solution of Uranine at a concentration of ~10 µg/L that had the same density as the seawater, and all the microcosms were well aerated during the incubation. After tracer addition, 1.5 ml water samples were taken by pasteur pipettes at 0, 2, 4, 22.5, 24 h and the Na-fluorescein concentrations were measured at 520 nm using a Turner Quantech Digital Fluorometer (FM 109530-33) with 490 nm as the excitation wavelength. Uranine fluxes were estimated from the decrease of Uranine over time in the water column. Faunal-mediated Uranine fluxes were quantified by subtracting Uranine fluxes measured in the abiotic control cores and regarded as proxy for bio-irrigation. Total biomass (gAFDW m^{-2}) of *H. diversicolor* and *L. balthica* individuals from each microcosm was measured at the end of each experiment.

4.2.3 Data analysis

4.2.3.1 Scaling density- and biomass-dependent effects on SCOC

Following ecological scaling theory (Brown et al., 2004), the mediating effect on sediment metabolism and sediment irrigation performed by a homogeneous species population

can be expressed as a power function of the individual body size M times the population abundance (number of individuals per unit surface) A: $Y = aM^bA$ (1), where faunal-mediated O_2 uptake and bio-irrigation (i.e. faunal-mediated Uranine flux) are the response variables (Y), M and A are the individual body size (mg AFDW) and the abundance (ind m^{-2}) of the examined animals respectively, a is the coefficient quantified in the model, and b is the allometric exponent relating the body size to the individual activity. In most aquatic ecosystems, the macro-ecological pattern of the abundance and normalized biomass spectra at the community level is observed to be linear (or near linear) with a slope close to -1 (Sprules and Barth, 2016). Previous studies revealed the necessity to consider not only body size and density, but also total biomass of the fauna when comparing bioturbation effects among fauna species (Kristensen et al., 2013). Thus, we also fitted the relationship between response variables (Y; faunal-mediated O_2 uptake and bio-irrigation) and the total biomass (W; gAFDW m^{-2}) and the equation is: $Y = cW^d$ (2), where c is the quantified coefficient and d is the biomass-dependent scaling exponent.

Taking the log of both the sides of equation (1) and (2), the equations can be rearranged in suitable form for multivariate log-log regression analysis:

$$\log (Y) = \log (a) + b \log(M) + \log (A)$$

$$\log (Y) = \log (c) + d \log (W)$$

4.2.3.2 Statistical analysis

Analysis of covariance (ANCOVA) was conducted to determine the habitat dependence of faunal-mediated O_2 uptake and bio-irrigation controlling for biomass (covariate). To match the linear ANCOVA assumptions, biomass of *H. diversicolor* and *L. balthica* in all experiment schemes, and the bio-irrigation from the *L. balthica* experiments at the muddy habitat was normalized via log transformation (Supplementary material Table

4.3). The bio-irrigation measurements from treatment 2 and 6 from the *L. balthica* experiment scheme in muddy habitats were excluded from the ANCOVA analyses analysis to achieve normality of residuals, since data were identified as outliers (Casewise diagnostics outlier outside 3 standard deviations, SPSS). In addition, the assumptions of the homogeneity of regression slopes and the homogeneity of variance were verified in faunal-mediated O_2 uptake and faunal-mediated Uranine fluxes (Supplementary material Table 4.3). Linear regression was used to determine to what extent the variance of faunal-mediated O_2 uptake can be explained by the bio-irrigation and respiration. The level for statistical significance was set at 0.05 in all analyse.

4.3 Results

4.3.1 Biomass- and density- dependence of faunal-mediated O_2 uptake and irrigation activity

Power equations for the prediction of O_2 uptake and Uranine flux mediated by the target organisms are presented in Table 4.3. Faunal-mediated O_2 consumption as a function of biomass follows a power law with a sublinear exponent (< 1) for each species in both habitats (Figure 4.3a, 4.3b). The relations between faunal-mediated Uranine fluxes and biomass follow similar power law scaling with sublinear scaling for *H. diversicolor* in both habitats with different sediment types (sandy and muddy) (Figure 4.4a). In contrast, Uranine fluxes versus biomass for *L. balthica* shows different power scaling patterns in the two sediment types, exhibiting a clear power law with sublinear exponent in sandy sediments while faunal-mediated Uranine fluxes scaled near linear (exponent=1.06; Figure 4.4b) as a function of biomass in muddy sediments.

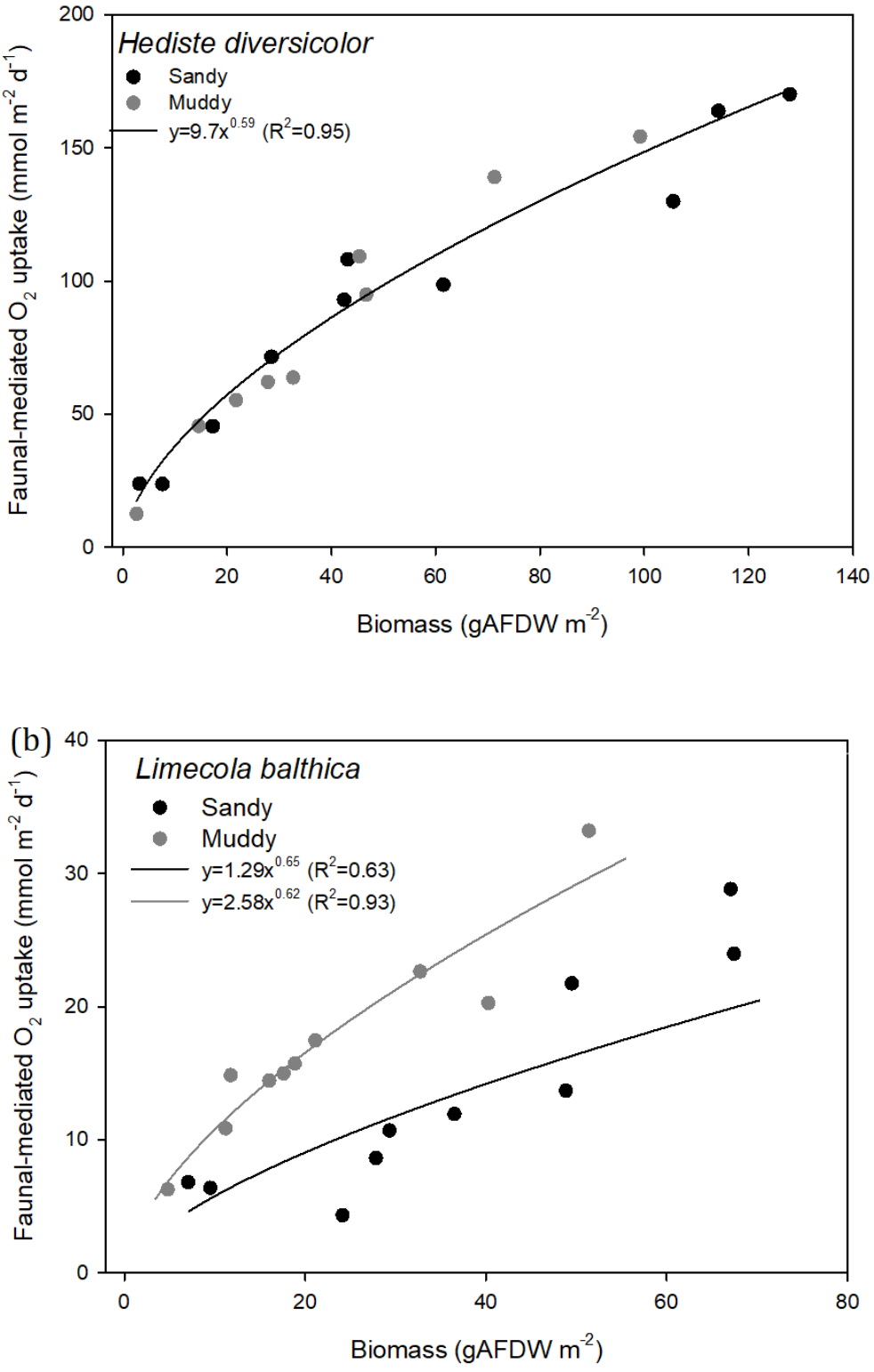


Figure 4.3 Faunal-mediated O_2 uptake (mmol m^{-2} d^{-1}) measured in each microcosm as a function of animal biomass (AFDW), and the best-fit power functions ($p<0.05$) for (a) *H. diversicolor* and (b) *L. balthica*.

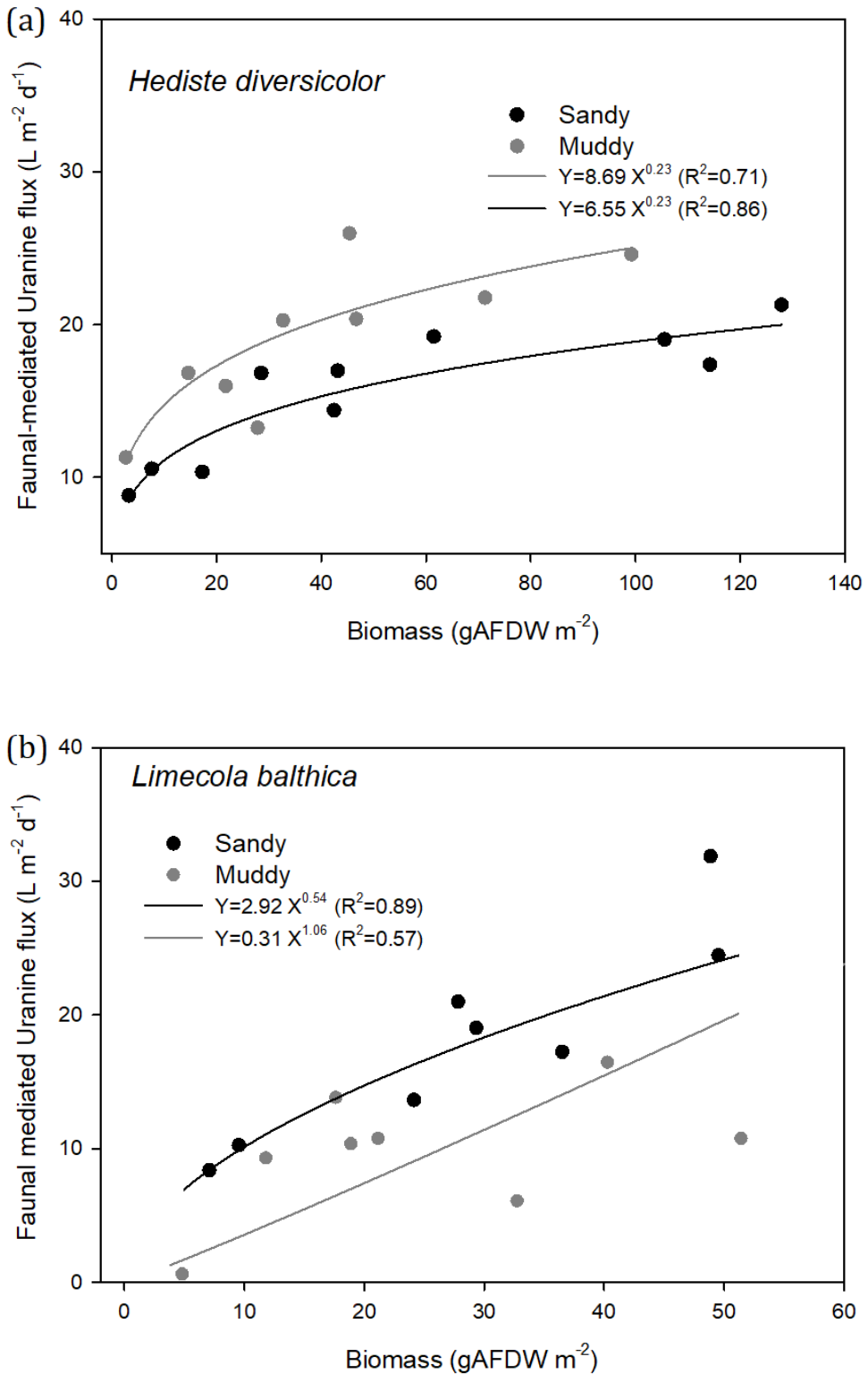


Figure 4.4: Faunal-mediated Uranine flux (L d m-^{2}) measured in each microcosm with the addition of the target organisms and the best-fit power functions ($p<0.05$) for (a) *H. diversicolor* and (b) *L. balthica*.

In general, power models as a function of biomass had better performance than models with body size and abundance as explanatory variables (Table 4.3a, 4.3b). Biomass models were able to explain over 90% of the observed variance in faunal-mediated O_2 uptake, except for the model of *L. balthica* in sandy habitat (R^2=63%; Table 4.3a). Allometric models based on per capita body size and individual's abundance explained 64% (sandy; *L. balthica*) to 88% (muddy; *H. diversicolor*) of the observed variance in faunal-mediated O_2 uptake (Table 4.3a). The body size exponent varied from 0.58-0.83 and the biomass exponents ranged from 0.58-0.71 (Table 4.3a).

The biomass model of *H. diversicolor* described 71% and 86% of the variability in faunal-mediated Uranine flux at muddy and sandy habitats respectively. For *L. balthica*, biomass was able to explain 57% and 89% of the observed variance in bio-irrigation in muddy and sandy habitats. However, no significant allometric fitting of bio-irrigation can be found based on the predictors body size and abundance in the two *H. diversicolor* experiment schemes (muddy and sandy habitats) and in the *L. balthica* experiment from the muddy habitat (Table 4.3b).

Table 4.3: Summary of the allometric models in predicting (a) Faunal-mediated O_2 uptake (mmol m^{-2} d^{-1}), (b) Faunal-mediated Uranine flux from sandy habitat and muddy habitat ($p<0.05$).

(a) Faunal-mediated O_2 uptake		=a(Bodysize)^{b}Abundance				=c(Biomass)^d			
Species	Habitat	a	b	R^2	Adj. R^2	c	d	R^2	Adj. R^2
L. balthica	Sandy	0.10	0.69	0.64	0.6	1.29	0.65	0.63	0.59
	Muddy	0.37	0.83	0.88	0.87	2.58	0.62	0.928	0.92
H. diversicolor	Sandy	0.39	0.58	0.75	0.72	9.75	0.58	0.955	0.95
	Muddy	0.90	0.78	0.86	0.85	6.25	0.71	0.99	0.98

(b) Faunal-mediated Uranine flux		=a(Bodysize)^{b}Abundance				=c(Biomass)^d			
Species	Habitat	a	b	R^2	Adj. R^2	c	d	R^2	Adj. R^2
L. balthica	Sandy	0.2	0.7	0.76	0.73	2.92	0.54	0.89	0.88
	Muddy					0.31	1.06	0.57	0.5
H. diversicolor	Sandy	No allometric fitting				6.55	0.23	0.86	0.84
	Muddy					8.69	0.23	0.71	0.68

4.3.2 Habitat effects on faunal-mediated O_2 consumption and bio-irrigation

Measures of O_2 uptake in the water column without the addition of macrofauna were 1.2 ± 0.2 mmol m^{-2} d^{-1}; whilst the O_2 consumption in the abiotic controls with muddy and sandy sediments added were 8.8 ± 2.3 mmol m^{-2} d^{-1} and 1.9 ± 0.3 mmol m^{-2} d^{-1} respectively (Supplementary material Table 4.2). The *L. balthica* treatments increased the O_2 uptake by factors between 4.2-11.1 in seawater and 1.6 - 14.2 in the sediment over the controls. The O_2 uptake in seawater with *H. diversicolor* addition were 5.7-153.6 times and 2.8-100.4 times higher than the background values measured in the control cores with sea water and sediments respectively. The Uranine fluxes quantified in the control cores from muddy habitats were 12.7 ± 0.17 L m^{-2} d^{-1} and 2.7 ± 1 L m^{-2} d^{-1} in sandy habitats (Supplementary material Table 4.2).

Faunal respiration of *H. diversicolor* measured in the polyhaline seawater fell in the range of 6.1-124.1 mmol m^{-2} d^{-1} corresponding to the abundance 943-5660 ind m^{-2} when combining both habitats (Supplementary Figure 4.1a). This amounted to a per capita respiration rate of *H. diversicolor* in the range of 0.007-0.07 mmol ind^{-1} d^{-1}. For *L. balthica* in both sandy and muddy sediments, faunal respiration ranged from 4.2-12.9 mmol m^{-2} d^{-1} corresponding to the abundance 934-5660 ind m^{-2} (Supplementary Figure 4.1b). The individual respiration rates ranged within 0.001-0.012 mmol ind^{-1} d^{-1}.

ANCOVA revealed that biomass significantly predicted faunal-mediated O_2 uptake by both species in both habitats ($p<0.05$; Table 4.4a, 4.4b). There was no significant difference between habitats in faunal-mediated O_2 uptake by *H. diversicolor* ($p=0.678$; Table 4.4a), whilst a significant habitat effect in faunal-mediated O_2 consumption by *L. balthica* was found ($p<0.05$; Table 4.4b) with more enhanced O_2 consumption per gram biomass in the muddy habitat. The faunal-mediated O_2 uptake by *H. diversicolor* was 2.6 ± 1.8 mmol $gAFDW^{-1}$ d^{-1} and 2.5 ± 0.9 mmol $gAFDW^{-1}$ d^{-1} in muddy habitat and sandy habitat respectively, which were larger in comparison to faunal-mediated O_2 uptake by *L. balthica* valued at 0.9 ± 0.3 mmol $gAFDW^{-1}$ d^{-1} in the muddy habitat and 0.4 ± 0.2 mmol $gAFDW^{-1}$ d^{-1} in the sandy habitat. Faunal-mediated Uranine fluxes quantified from *L. balthica* treatments fell within the ranges of 8.4-31.9 L m^{-2} d^{-1} and 0.6-16.5 L m^{-2} d^{-1}, resulting in the bio-irrigation of 0.7 ± 0.5 L $gAFDW^{-1}$ d^{-1} and 0.5 ± 0.4 L g $AFDW^{-1}$ d^{-1} in sandy and muddy sediments respectively; whilst the bio-irrigation measured from *H. diversicolor* treatments were 8.8-21.3 L m^{-2} d^{-1} in sandy habitat and 11.3-26 L m^{-2} d^{-1} in muddy sediments. The faunal-mediated Uranine fluxes by *H. diversicolor* valued at 0.7 ± 0.8 L $gAFDW^{-1}$ d^{-1} in sandy habitats and 1 ± 0.9 L $gAFDW^{-1}$ d^{-1} in muddy habitats.

Table 4.4: ANCOVA results in species subsets (a) *H. diversicolor* and (b) *L. balthica*. Faunal-mediated O_2 uptake is the dependent variable, Habitat is the factor, and biomass is the covariate. Once the significant effect of habitat was found in *L. balthica* dataset, the estimated marginal means were analysed.

(a) *H. diversicolor*	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36660.8	2	18330.4	52.8	<0.001
Intercept	4416.7	1	4416.7	12.7	0.0
biomass	36228.7	1	36228.7	104.4	<0.001
habitat	61.8	1	61.8	0.2	0.7
Error	5897.8	17	346.9		
Total	197874.5	20			
Corrected Total	42558.6	19			
R Squared = 0.86 (Adjusted R^2 = 0.85)					

(b) *L. balthica*	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	991.2	2	495.6	50.4	<0.001
Intercept	67.5	1	67.5	6.9	0.018
biomass	934.4	1	934.4	95.1	<0.001
habitat	344.1	1	344.1	35.0	<0.001
Error	167.1	17	9.8		
Total	5886.0	20			
Corrected Total	1158.4	19			

R Squared = 0.86 (Adjusted R^2 = 0.84)

Estimated marginal means

Sediment types	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Sandy	10.9	1.0	8.7	13.1
Muddy	19.9	1.0	17.7	22.0

The biomass-dependent Uranine flux by *H. diversicolor* was higher in muddy habitat as compared to sandy habitat ($p<0.05$; Table 4.5a). The bio-irrigation variability explained by biomass of *H. diversicolor* were 86% and 71% in muddy and sandy habitats ($p<0.05$; Figure 4.4a). Biomass of *L. balthica* was found to be a significant predictor for bio-irrigation accounting for 57% and 89% of the bio-irrigation variability in muddy and sandy habitats respectively ($p<0.05$; Figure 4.4b), and a significant difference was detected in the biomass-dependent Uranine fluxes mediated by *L. balthica* between two habitats ($p<0.05$; Table 4.5b).

Faunal-mediated O_2 uptake by *H. diversicolor* in both habitats (sandy and muddy) increased

significantly ($p<0.05$; Figure 4.5a) with increasing irrigation intensity. For *L. balthica* in sandy

sediments, a significant positive relationship was found between faunal-mediated O_2 and bio-irrigation ($p<0.05$; Figure 4.5b) whilst in muddy sediments the variation in faunal-mediated O_2 uptake was not linearly related to irrigation activities.

Table 4.5: ANCOVA results in species subsets (a) *H. diversicolor* and (b) *L. balthica*. Faunal-mediated Uranine flux is the dependent variable, Habitat is the factor, and biomass is the covariate. The estimated marginal means were computed when sediment effect was found significant. Uranine fluxes mediated by *L. balthica* were log transformed.

(a) *H. diversicolor*	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	343.9	2	172	29.5	<0.001
Intercept	48.7	1	48.7	8.4	0.01
biomass	272.7	1	272.7	46.8	<0.001
habitat	91	1	91	15.6	0.001
Error	99	17	5.8		
Total	6476.2	20			
Corrected Total	442.9	19			
R^2 = .78 (Adjusted R^2 = 0.8)					
Estimated marginal means					
Habitat	Mean	Std. Error	95% Confidence Interval		
			Lower Bound	Upper Bound	
Sandy	15.2	.8	13.6	16.8	
Muddy	19.5	.8	17.9	21.1	

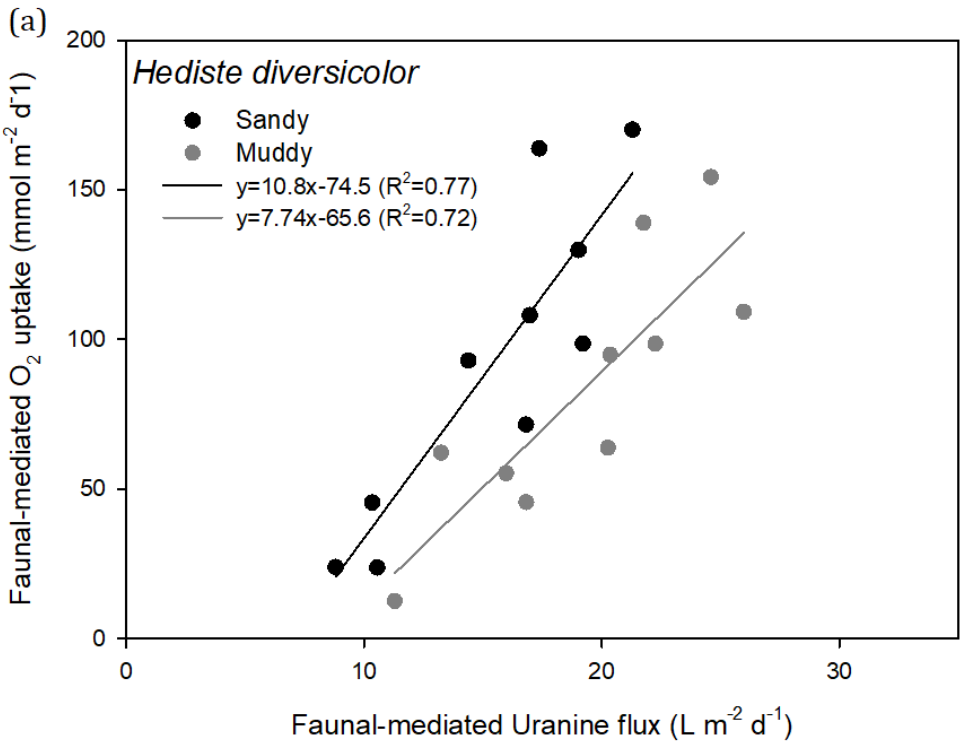
(b) *L. balthica*	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.7	2	0.9	16.5	<0.001

Intercept	8.6E-5	1	8.6E-5	0.002	0.97
biomass	1	1	1	19.6	<0.001
habitat	0.3	1	0.3	5.6	0.031
Error	0.8	15	0.05		
Total	23.9	18			
Corrected Total	2.5	17			

R^2 = 0.687 (Adjusted R^2 = 0.6)

Estimated marginal means

Habitat	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Sandy	1.2	0.07	1.1	1.4
Muddy	0.9	0.08	0.8	1.1



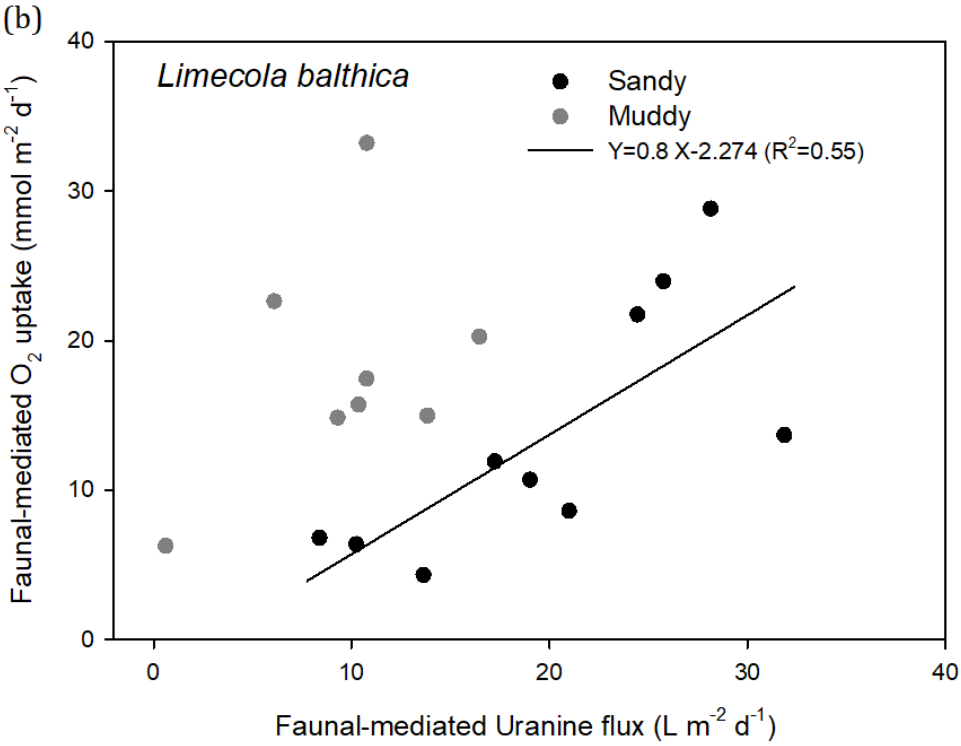


Figure 4.5: Relationship between faunal-mediated O_2 uptake and faunal-mediated Uranine fluxes by (a) *H. diversicolor* and (b) *L. balthica.* Relationship between faunal-mediated O_2 uptake and faunal-mediated Uranine fluxes by (a) *H. diversicolor* and (b) *L. balthica.* The significant linear fitting was found in *H. diversicolor* dataset in both sandy and muddy habitats ($p<0.05$).

4.4 Discussion

4.4.1 Density- and biomass-specific faunal effects on sediment O_2 consumption

The positive relationship between macrofauna population density and O_2 uptake has been shown in laboratory experiments under constant conditions (Papaspyrou et al., 2005). We also found that population density of macrofauna can significantly predict faunal-mediated O_2 uptake and bio-irrigation, and a power function of biomass was verified to quantify these relationships (Table 4.3a, 4.3b). The estimated body size scaling exponents of faunal-mediated O_2 uptake were below 1 (b=0.58-0.83; Table 4.3a, 4.3b), indicating that the faunal-mediated O_2 uptake with increasing body size is allometric

(Wen and Peters, 1994). This allometric relation is probably linked to respiration, which scales with the 3/4 power of body size, implying that the individual metabolism per volume scales with the surface area (Brown et al., 2004). Our results correspond with the previous study that body size mediates the role of animals in nutrient cycling in aquatic ecosystems (Hall et al., 2007). The biomass (AFDW) exponents quantified in the *H. diversicolor* experiment scheme ranged from 0.58-0.71 (Table 4.3a), which is in accordance with the relationships quantified between the rate of oxygen consumption and body weight (dry weight) monitored in eight marine polychaetes with the exponent falling in the range 0.61-0.69 (Shumway, 1979).

In comparison to body size and abundance as predictors, a higher proportion of the variance in faunal-mediated O_2 uptake was explained by total biomass (R^2=0.63-0.99) (Table 4.3a). The lower predictive power of the body size model in faunal-mediated O_2 consumption (Table 4.3a), together with the inadequacy of body size and abundance as significant predictors for bio-irrigation by *H. diversicolor* in both habitats and *L. balthica* in the muddy habitat (Table 4.3b), suggested that the per capita/body size effect in O_2 and Uranine fluxes cannot be simply added and there exist nonlinear density-dependent effects on solute exchange. These might be induced by individual behaviours, including the frequency and intensity of bio-irrigation, such that the increase in species density leads to a decrease in the individual fluxes (Matisoff and Wang, 2000; Reible et al., 1996). The disproportional use of metabolic energy in ecosystem engineering differs between size classes (Cozzoli et al., 2018), which might explain why body size is an inadequate predictor. Smaller individuals invest relatively more energy in ecosystem engineering, thus organisms with various body size in one experiment scheme might lead to different disproportional scaling effects. Thus, it is difficult to derive a shared scaling exponent across different body sizes. In addition, individuals may interfere with each other in the

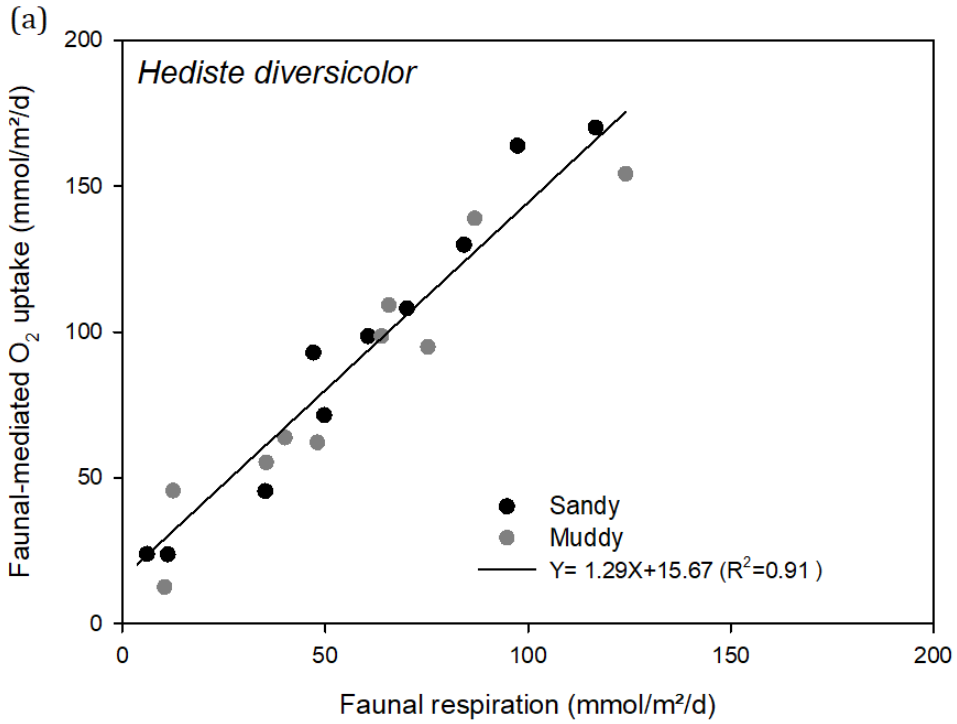
small space, and the influence areas overlap more for motile organisms such as *H. diversicolor* in comparison to more sedentary organisms such as *L. balthica* (Van Prooijen et al., 2011). In scaling body size and metabolic rate, the changes in constants (a and c in the allometric functions) are related to the different use of energy by different organisms' groups (Ernest et al., 2003). In this study the quantified constants a and c in allometric scaling of faunal-mediated O_2 uptake and bio-irrigation exhibited consistent distinction in different species, characterized by higher values in *H. diversicolor* models compared with *L. balthcia* models (Table 4.3a, 4.3b). Therefore, it can be expected that the target organisms showed different functionalities not only in stimulating O_2 fluxes, but also in mediating solute transfer (i.e. bio-irrigation).

4.4.2 Context-dependency of bioturbation functional group-influence on O_2 fluxes

Our experimental results of total respiration were comparable with sediment community O_2 consumption measurements of complete macrobenthic communities at the same locations (polyhaline zone of the Scheldt estuary) at a similar temperature (**Chapter 2-3**). Faunal-mediated O_2 uptake in this study aligned with weight-specific conversion factors of flux enhancement by *H. diversicolor* (Banta et al., 1999; Christensen et al., 2000; Kristensen and Hansen, 1999; Kristensen et al., 2011) and the observed O_2 fluxes enhanced by *L. balthica* found in the St-Lawrence estuary (52.8±1.18 mmol m^{-2} d^{-1} at the density of 3000 ind m^{-2}; St-Lawrence estuary) (Michaud et al., 2005).

The increase of benthic metabolism by burrow dwelling and irrigating infauna is considered a consequence of enhanced microbial activity, altered degradation pathways and the faunal respiration of the infauna itself (Aller and Aller, 1998; Glud, 2008). We measured that ~60 % of the faunal-mediated O_2 uptake could be attributed to respiration, therefore the remaining ~40 % was ascribed to the mediating effect of bio-irrigation and

particle mixing. However there might be an overestimation in macrofaunal respiration because the respiration data obtained in measuring O_2 uptake in sea water might be higher due to stress than actual animal respiration in the burrows (Vopel et al., 2003). Faunal respiration measured in the seawater is often regarded as a small fraction (<40%) of faunal-mediated O_2 consumption (Kristensen, 2000; Quintana et al., 2011), however it had a dominant effect on faunal-mediated O_2 consumption in this study and significantly predicted the faunal-mediated O_2 uptake in both species datasets encompassing sandy and muddy habitats (Figure 4.6a, 4.6b; $p<0.05$).



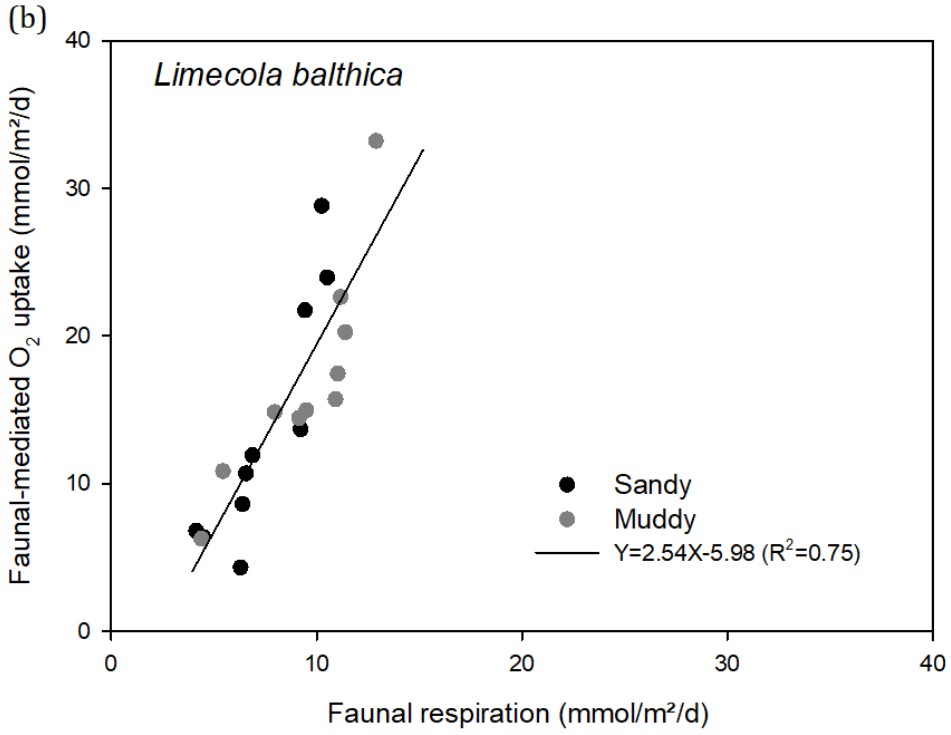


Figure 4.6: Relationship between faunal-mediated O_2 uptake by (a) *H. diversicolor* and (b) *L. balthica* and the correspondent faunal respiration. The significant linear fitting was found in *H. diversicolor* dataset encompassing both sandy and muddy habitats ($p<0.05$).

(a) Species-specific biological traits

The biomass-dependent enhancement in O_2 consumption was more pronounced for *H. diversicolor* than for *L. balthica* (Figure 4.3a, 4.3b), and it corroborates with previous findings that gallery-burrowing polychaetes affected benthic fluxes more than biodiffusing bivalves (Michaud et al., 2005). Pelegrí and Blackburn (1995) observed not only aerobic respiration but also nitrite fluxes generated by polychaetes to be ~2 times more than for the bivalves. The different responses of faunal-mediated O_2 uptake to increasing density and biomass of *H. diversicolor* and *L. balthica* might depend on their living positions, as *L. balthica* is mainly located at the top of the sediment and remains rather stationary in comparison to the polychaetes. Besides, it might also be attributed to different modes of bioturbation from *H. diversicolor* and *L. balthica*, the importance of

which has been demonstrated in organic matter mineralization (Mermillod-Blondin et al., 2004). The markedly different pumping behaviours were displayed by the tested species: the pumping activity of the *H. diversicolor* causes a tail-to-head directed ventilation current through its tube resulting in an in upward flow of oxygenated water in the sediment in front of the head, which is termed "piston pumping" (Riisgård and Larsen, 2005); whilst irrigation patterns of tellinid bivalves *L. balthica* were characterized by significantly shorter separation of irrigation bouts, which resulted in more continuous oxygenation of the sediment (Volkenborn et al., 2012).

(b) Environmental factor influences bioturbation effects within species

Abiotic sediment characteristics such as the permeability of the sediment and O_2 constraints also have an impact on the bioturbation modes (Glud, 2008; Koretsky et al., 2002). For *H. diversicolor*, we found higher bio-irrigation rates in muddy compared to sandy sediments (Figure 4.4a) with a significant habitat effect (ANCOVA, $p<0.05$; Table 4.5a). The finding of higher bio-irrigation in muddy sediments is in accordance with the community bio-irrigation measured in the baseline survey (June 2016; 20 °C) at the same study sites where *H. diversicolor* was identified as the dominant contributor (**Chapter 2-3**). This might be explained by the difference in oxygenation of permeable sandy sediments compared to muddy sediments where the low permeability limits advective pore water transport (Hedman et al., 2011). In the latter, their metabolic need for O_2 can be satisfied only by ventilation of their burrows which penetrate deeply into the anoxic zone (Gust and Harrison, 1981). As measured in the baseline survey (**Chapter 2-3**), O_2 penetrated indeed less deep in the cohesive sediment of the mudflat (muddy habitat) in comparison to the permeable sediment of the sandflat (sandy habitat) (Supplementary material Figure 4.3). Moreover, burrowing polychaetes such as *H. diversicolor* are able to move faster in sand than in mud (Scaps, 2002) and *H. diversicolor* tends to dig deeper into

sandy than muddy sediments (Esselink and Zwarts, 1989). Thus, the energy constraints on burrowing in sand may be less than those in mud. However, the more intensive bio-irrigation at the muddy habitat was not translated into higher O_2 fluxes in our experiment, for which no significant habitat influence was found (Table 4.4a, Figure 4.3a). For *H. diversicolor*, the finding presented in this study that per biomass (g AFDW m^{-2}) there is similar faunal-mediated O_2 uptake implies a potential of the broad application in extrapolating mediated O_2 consumption by *H. diversicolor* to a larger spatial scale (**Chapter 5**).

An opposite pattern was observed in *L. balthica*, i.e. a higher bio-irrigation and lower faunal-mediated O_2 uptake at the sandy compared to the muddy habitat (Figure 4.4b) whilst the habitat difference in faunal-mediated O_2 uptake (Table 4.4b) and bio-irrigation (Table 4.5) was significant. At the sandy habitat, food availability in the water column (Hummel, 1985; Lin and Hines, 1994) might play a role in more intensive bio-irrigation for *L. balthica*, since there is less organic carbon available in sandy sediments compared to muddy sediments (Supplementary material Figure 4.3). Therefore *L. balthica* might have to feed more thereby stimulating sediment irrigation through the pumping and release of water through exhalent siphon in the subsurface (Volkenborn et al., 2012). Finally the stronger faunal-mediated O_2 consumption by *L. balthica* at the muddy habitat can probably be explained by the re-oxidation of reduced substances in the anoxic subsurface of the muddy sediment; Indeed, no significant linear relationship was found between *L. balthica*-mediated irrigation and oxygen consumption suggesting the importance of other governing mechanisms. Henriksen et al. (1983) and Michaud et al. (2006) found highly variable effects of *L. balthica* on benthic N cycling, corroborating the found context-dependent influence of *L. balthica* on sediment metabolism in this study.

4.5 Conclusion

The burrowing polychaete *H. diversicolor* and bivalve *L. balthica* offer an ideal model system to examine the role of macrobenthic bioturbators in mediating O_2 uptake in estuarine sediment. Our results showed that a major portion of the variance in sediment oxygen uptake and bio-irrigation can be explained by the per capita body size and abundance, therefore we confirmed our first hypothesis that allometric principles of metabolism scaling with population density can be applied to quantify the faunal effects on sediment O_2 uptake. Total biomass of the inhabiting bioturbators appeared to be a better predictor in assessing O_2 uptake and bio-irrigation than population density. Respiration was significant in predicting faunal-mediated O_2 uptake among different species (*H. diversicolor* and *L. balthica*) across different habitats (sandy and muddy sediments); whilst the relationship between bio-irrigation and faunal-mediated O_2 uptake was only significant in *H. diversicolor* experiment schemes and for *L. balthica* in sandy sediments, with a lower predictive power compared to respiration as the predictor. Overall, the extent to which the variance of faunal-mediated O_2 uptake can be explained is also conditioned by the interplay between the abiotic environment and the biological traits of the specific species, which is in support of the second hypothesis that biological traits (e.g. bio-irrigation) and sediment physico-chemical properties significantly affect faunal-mediated O_2 consumption.

Acknowledgements

This work was funded by the MARES doctoral programme (2012-1720/001-001-EMJD) and EMBRC Belgium – FWO project GOH3817N. We appreciate the valuable support of all colleagues from Ghent University, Marine biology group, who helped in field sampling and laboratory analyses, in particular laboratory technicians Dirk Van Gansbeke and Bart

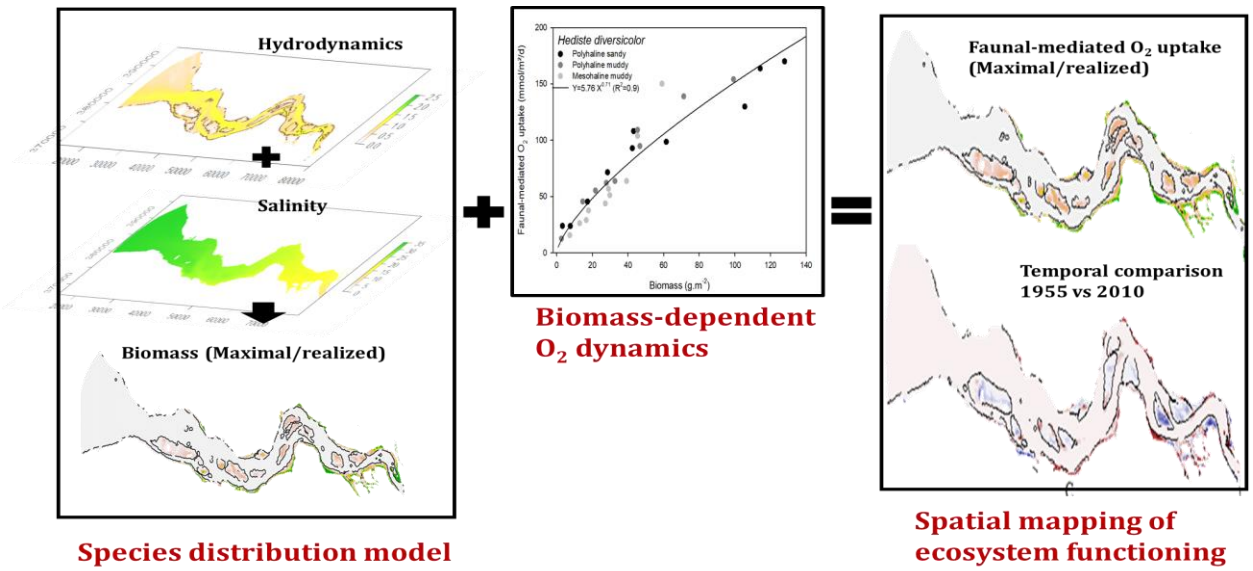
Beuselinck, and special thanks to An-Sofie D'Hondt, Tim Tkint, and the students of the IMBRSea programme of UGent for their help during the field sampling.

Chapter 5 A benthic perspective in assessing estuarine ecosystem management: modelling *H. diversicolor* influence on O_2 uptake in the Western Scheldt (1955 vs 2010)

Abstract

As global anthropogenic pressures on natural environments continue to grow, the development of assessment tools for ecosystem-based monitoring and management is becoming increasingly important and management focus shifts to addressing issues of loss of function and concomitant ecosystem services. In this study, we quantified the contribution of the sediment-dwelling polychaete *H. diversicolor* across a range of population densities in sediment metabolism and extrapolated the spatial variability of the faunal-mediated O_2 consumption based on its biomass and distribution within its natural habitats along the Western Scheldt. Biomass-scaling of faunal-mediated O_2 by *H. diversicolor* was quantified from laboratory-controlled microcosms containing defaunated sediment with artificially composed faunal densities in its habitats (polyhaline sandy sediment, polyhaline muddy sediment and mesohaline muddy sediment) along the estuarine gradients of the Western Scheldt; the spatial variability of the faunal-mediated O_2 consumption was extrapolated at landscape scales by integrating spatial distribution mapping of *H. diversicolor* derived from a multi-quantile regression model. The potential (habitat suitability) and realistic biomass of *H. diversicolor* were predicted by environmental drivers (i.e. hydrodynamics and salinity); two temporal scenarios for the years 1955 and 2010 were compared to investigate changes in faunal-mediated O_2 consumption by *H. diversicolor* in relation to the recent anthropogenic modifications of the estuary. Biomass of *H. diversicolor* was a highly significant and low

variability (R^2 = 0.9) determinant of faunal-mediated O_2 uptake ($F_{1,26}$ = 119.6, $p < 0.001$) that was unaffected by habitat type ($F_{2, 26}$ = 3.12, p = 0.06). Therefore, the biomass-dependent function in quantifying faunal-mediated O_2 uptake can be shared across different habitat types to quantify faunal-mediated O_2 uptake. The maximum faunal-mediated O_2 uptake estimated from potential biomass of *H. diversicolor* was 153.4 mmol m^{-2} d^{-1} in both 1955 and 2010, and the predictions for the Western Scheldt suggested a total biomass of 4407.12 kg and 2915.03 kg, and O_2 consumption of 8772 and 6201 mol d^{-1} for 1955 and 2010 respectively, representing a reduction of 34% and 29% in these metrics over this time period. The realistic distribution of *H. diversicolor* revealed a decline in total biomass of 65.8% (from 3044.71 kg to 1040.03 kg) in the Western Scheldt, which corresponded with a 60.3% reduction in the faunal-mediated O_2 consumption in the whole estuary from 5401.31 mol d^{-1} to 2145.93 mol d^{-1}. Overall, hydrodynamic changes induced by anthropogenic activities (i.e. dredging) are most likely to negatively affect the spatial distribution and biomass of *H. diversicolor*, whilst numerous indirect effects are likely to reverberate throughout the ecosystem. This approach could be used to predict faunal-mediated O_2 consumption over large benthic landscapes at high spatial resolution caused by changes in population density of benthic bioturbators, further estimate ecosystem-scale changes for managing suitability over large temporal scale.



Graphical abstract of Chapter 5

This Chapter is in preparation as:

A benthic perspective in assessing estuarine ecosystem management: modelling H. diversicolor influence on O_2 uptake in the Western Scheldt (1955 vs 2010)

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5.1 Introduction

Soft-sediment environments are some of the most widespread habitats on the planet incorporating most of the world's coastal zones and estuaries (Snelgrove, 1999). Marine soft-sediments are especially important, which account for ~2/3rd of global ecosystem services (Snelgrove, 1997) via ecosystem functions and processes, such as organic matter remineralization, primary production, sediment transport, and nutrient cycling. These processes are often measured as proxies for ecosystem health and functioning and indicators of environmental change (Thrush et al., 2006; Webb and Eyre, 2004; Widdicombe and Austen, 1998). Marine environments and especially the coastal margin is under increasing threat from anthropogenic activities (Firth et al., 2016; Knights et al., 2015). Human disturbance is an important driver of loss of biodiversity (Loreau, 2010) and ecosystem services (Knights et al., 2014; Lemasson et al., 2017).

Macrobenthic species play a pivotal role in the provision of services from sedimentary ecosystems not only as the important components of food webs (Herman et al., 1999b), but also in mediating estuarine biogeochemistry through their burrowing, ventilation, ingestion and defecation activities (Heip et al., 2001). Their activities can result in valuable ecosystem services such as soil formation, soil fertility, oxygen and water regulation (de Bello et al., 2010; De Valença et al., 2017; Wilkinson et al., 2009). Macrobenthos actively mix the sediment, so that the grain size distribution is modified (Giangrande et al., 2002) and the structure, porosity and erodibility of the sediment are changed (Rowden et al., 1998; Widdows et al., 2000). Ventilation activities and flushing of burrows redistribute nutrients (Lohrer et al., 2004b) and oxygen (Aller, 1982), contributing to the oxygenation of sediments and enhancing the nutrient availability for primary production and biogeochemical cycling (Blackburn, 1988; Braeckman et al.,

2014a). In coastal seas, these processes can supply up to half the nutrients for primary production (Lohrer et al., 2004b).

The biodiversity of benthic communities can change in association with variability in the hydrodynamic, morphological and chemical features of estuarine environments (Cozzoli et al., 2017; Esselink and Zwarts, 1989; Thrush et al., 2003; Ysebaert et al., 2003, 2002) leading to marked differences in ecosystem functioning depending on location (Lohrer et al., 2004a; Sandwell et al., 2009). For example, increased organismal densities can lead to greater availability of metabolic products such as NH_4^+, which stimulate microbial metabolism that alter nitrification/denitrification rates (Henriksen et al., 1983; Kristensen, 1985). Consequently, to better understand the contribution of soft-sediment landscapes (e.g. an estuary) to ecosystem functioning requires not only understanding of distributional patterns but also variation in the functioning of those organisms across that landscape (Suding et al., 2008; Williamson et al., 1999).

The extrapolation of benthos-mediated ecosystem functioning to a landscape scale can be difficult due to the uncertainty of the relative contribution of benthic species to the geochemical processes within the high heterogeneity at a variety of spatial and temporal scales (D'Andrea and DeWitt, 2009). A number of recent studies have shown that the relationship between macrobenthos and ecosystem functioning can be context-dependent in aquatic ecosystems (Mermillod-Blondin and Rosenberg, 2006; Tylianakis et al., 2008). For example, the effect of bioturbation and its importance vary depending on hydrological characteristics of the sediment systems (Queirós et al., 2011). This may be confounded, for instance, by some bioturbating benthic species being able to facultatively switch between feeding modes under differing environmental regimes (Marinelli and Williams, 2003), which could mislead our interpretation of functioning if unknown (Tang and Kristensen, 2007). However, few studies have considered that single

species can perform differing ecosystem functions dependent on their sediment environments, and the interactions between habitat, organism behaviour and density can infer changes to the ecosystem processes they generate (Escapa et al., 2008; McCraith et al., 2003). In this study we not only estimated the role of biomass and environmental context (salinity and sediment types) but also the context-specific functioning of organisms, to encompass between-estuaries variation from the target species' response. Therefore, the biological and environmental factors and their interaction in influencing ecosystem functioning at a broad scale can be assessed. The relationship is hypothesized to be shared across abiotic gradients; tested by measuring its faunal-mediated O_2 consumption. Quantifying density-dependent effects of bioturbators on sediment biogeochemistry is a necessary starting place for characterization of species-specific contributions, following which, data can be extrapolated to provide landscape-scale estimates using species distribution modelling. Regression-based species distribution models can forecast/hindcast biological response to environmental change at different space and time scales even when the underlying mechanisms are complex and difficult to unravel (Grand and Cushman, 2003; Ysebaert and Herman, 2002). In this study, temporal variation of the benthos-mediated oxygen consumption at estuarine landscape scales was assessed by integrating the density-dependent ecosystem functioning models with species distribution prediction in different years.

As global anthropogenic pressures on natural environments continue (Halpern et al., 2008; Knights et al., 2015), the development of assessment tools to support ecosystem-based monitoring and management is increasingly important, especially given the recent shift in management focus toward addressing loss of function and concomitant ecosystem services (Knights et al., 2014; Needham et al., 2011). The upscaling of quantitative descriptions of ecosystem functioning (see **Chapter 4**) to a landscape scale

provides an estimate of the total value of the faunal-mediated O_2 uptake; here O_2 consumption in the sediment is used as a tracer for biological activities and an indicator of the biological status of the environment and the cycling of elements (Canfield et al., 2005). The functioning of the burrowing polychaete, *Hediste diversicolor*, was specifically considered as it is regarded as one of the most effective oxygenators in altering oxygen dynamics via its feeding behaviour, sediment reworking activities, and a major link in food webs for fish and birds (Bocher et al., 2014; Philippart et al., 2007; Vinagre et al., 2012). We quantified the contribution of *H. diversicolor* to sediment metabolism across a range of population densities and extrapolated the spatial variability of the faunal-mediated O_2 consumption based on its abundance and distribution within its natural habitats, using the Scheldt estuary as a case study.

5.2 Materials and Methods

5.2.1 Study area

The Western Scheldt estuary in the SW of the Netherlands connects the Scheldt River in Belgium to the North Sea (Figure 5.1) and is essential for the economy by providing a navigable channel for the harbour of Antwerp. To maintain access, the channel is dredged (currently between 6.5 - 7 x 10^6 m^3 of sediment annually; Cozzoli et al., 2017), but the frequency of dredging has intensified since the 1960s due to a continued increase in the number and size of container vessels. Dredging dramatically alters tidal water movement, the tidal range, and in the Scheldt has seen increased mean tidal current velocity of ~30% since 1955 (Smolders et al., 2013).

The Western Scheldt is of high ecological importance. It is a multi-channel system with several tidal flats located in middle and along its edges, which are inhabited by a rich diversity of benthic organisms (Wang, 2015). It has a salinity gradient including

polyhaline and mesohaline conditions which strongly influence the benthos composition of the tidal flats. It has been recognized that economic growth typically requires activities that have a negative impact on safety and its ecology (dredging and harbour extensions), thus the establishment of the legal and policy frameworks supporting the maintenance of ecological coherence and connectivity has been identified as being necessary for biodiversity conservation (Knights et al., 2015; Pearson et al., 2016). In the Scheldt estuary, the river basin management plans 2016-2021 are based on two European Directives (i.e. the Water Framework Directive and the Floods Directive). Thus, integration of ecological knowledge and economic forecasts is required to achieve effective ecosystem-based management in order to ensure the estuary benefits both humans and nature through the provision of ecosystems services (Knights et al., 2014).

5.2.2 Modelling faunal-mediated O_2 uptake at estuarine landscape level

Step 1: Experimental measurements and biomass-scaling of faunal-mediated O_2 uptake

(a) Target organism and experimental design

The value of *H. diversicolor* has been highlighted in estuarine ecosystem management due to its great effects on sediment biogeochemical processes and being biotic indicators of environmental issues (e.g. organic pollution) (Alla et al., 2006; Esselink et al., 1989; Scaps, 2002), due to its efficient physiological adaptation to variation of environmental factors such as salinity, temperature, oxygen, and sediment types (Fritzsche and von Oertzen, 1995; Murray et al., 2017; Smith, 1964). The dominant bioturbators can have the greatest per-individual effect on sediment turnover or bio-irrigation by virtue of having high individual biomass (Solan et al., 2004a) or the largest burrow galleries (Ieno et al., 2006; Norling et al., 2007). In the Scheldt estuary, *H. diversicolor* occupies habitats under a range of environmental conditions including polyhaline to mesohaline salinity zones and

contributes substantially to the total biomass of the benthic assemblage (Ysebaert et al., 2005, 1998). Another important role - the mediation of biogeochemical fluxes via bioturbation (e.g. particle reworking and bio-irrigation) - has recently been demonstrated in the Scheldt estuary (Fang et al., 2019).

As revealed in **Chapter 4**, the governing function of biomass scaling can be used to scale the biomass-dependent faunal mediated O_2 uptake of *H. diversicolor* to two different habitat types in the polyhaline zone. This implied a potential of the broad application in extrapolating mediated O_2 consumption by *H. diversicolor* to a larger spatial scale. To explore the potential of upscaling *H. diversicolor* bioturbation effect to estuarine ecosystem landscape level, a subset of the experimental results in **Chapter 4** was extracted which is composed of polyhaline sandy and muddy habitats. To ensure a full spatial coverage of natural habits of *H. diversicolor* in the Western Scheldt estuary, an extra experiment was performed to measure faunal-mediated O_2 by *H. diversicolor* in mesohaline muddy habitats, following the same protocol in **Chapter 4**.

Overall, the sampling locations of *H. diversicolor* habitats were stratified based on the Benthic Information System database (BIS) at NIOZ Monitor Taskforce (Netherlands), which contains macrobenthic distribution records collected since 1960 in the study region. *H. diversicolor* is most abundant in sandy and muddy sediments in the polyhaline zone, and in muddy sediments in the mesohaline zone (Ysebaert et al., 2003, 1998). The sedimentology (e.g. mud and sand content) and hydrodynamic forcing are the key features used to define high- (sandy) and low-dynamic (muddy) ecotopes (Twisk, 2002; van der Wal et al., 2017). Polyhaline locations with sandy and muddy sediments were selected based on their proximity to each other and similar physical water column properties including such as O_2 concentration (Van Ryckegem et al., 2014). Three locations with these environmental criteria were identified (Figure 5.1): (i) polyhaline

sandy habitat (51° 21' 00.2" N, 3° 43' 54.9" E) on the Paulina intertidal flat on the southern bank of the estuary; (ii) polyhaline muddy habitat (51° 20' 57.1" N, 3° 43' 35.4" E) on the Paulina mudflat close to the tidal marsh, and (iii) mesohaline muddy habitat on Groot Buitenschoor intertidal flat (51° 22' 29.6" N, 4° 14' 34.9" E) habitats.

Thirty microcosms with the addition of *H. diversicolor* were set up and used to simulate the environmental conditions found in the Scheldt (i.e. ten per habitat type: polyhaline sandy habitat, polyhaline muddy habitat, and mesohaline muddy habitat). Each microcosm was populated with *H. diversicolor* at a biomass found in nature (3.21-127.89 gAFDW m^{-2} in polyhaline sandy habitat; 2.66-99.26 gAFDW m^{-2} in polyhaline muddy habitat; and 7.32-59.04 gAFDW m^{-2} in mesohaline muddy habitat; Table 5.1) and faunal-mediated O_2 uptake was quantified following the experiment protocol in **Chapter 4**.

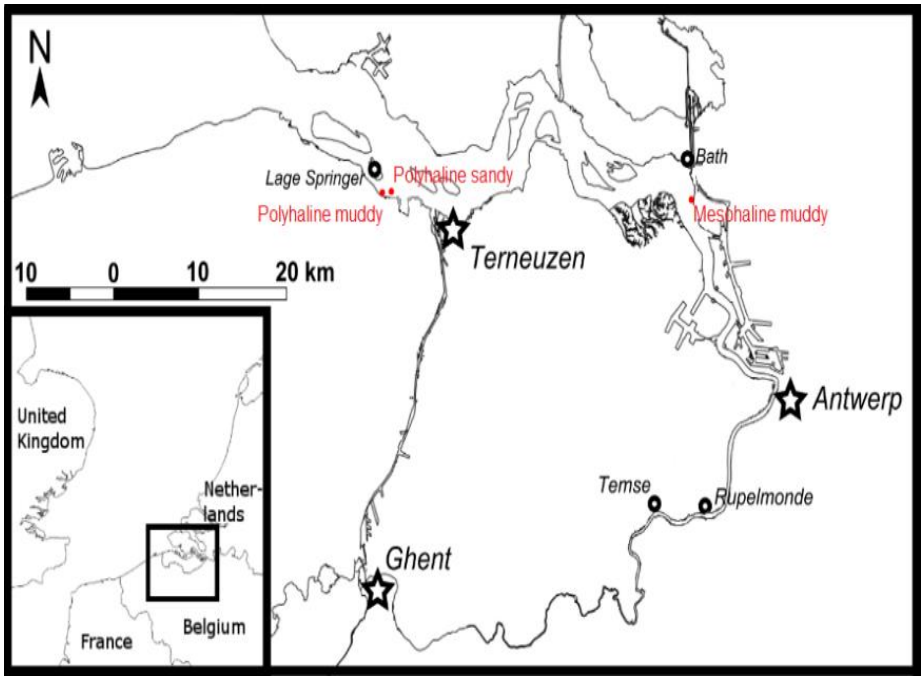


Figure 5.1: Sampling sites of *H. diversicolor* habitats in Western Scheldt: polyhaline sandy habitat, polyhaline muddy habitat, and mesohaline muddy habitat.

Table 5.1: Table of treatment design in three experiment schemes, which were in accordance with three natural habitats for *H. diversicolor* in the Western Scheldt. Several combinations of individual abundances and individual body sizes of target organisms *H. diversicolor* were tested according to their natural range of density levels in polyhaline sandy habitat, polyhaline muddy habitat, and mesohaline muddy habitat. Abundance in ind m^{-2}, biomass in g AFDW m^{-2}, body size in mg AFDW ind^{-1}.

Habitat	Polyhaline sandy			Polyhaline muddy			Mesohaline muddy		
Treatment	Abundance	Biomass	Body size	Abundance	Biomass	Body size	Abundance	Biomass	Body size
1	943	7.6	8.06	943	2.66	2.82	943	12.87	13.64
2	943	28.51	30.22	943	14.59	15.47	943	17.8	18.87
3	943	43.11	45.7	943	45.37	48.09	1887	7.32	3.88
4	1887	17.27	9.15	1887	27.82	14.74	1887	27.28	14.46
5	1887	3.21	1.7	1887	21.74	11.52	2830	39.23	13.86
6	2830	105.56	37.3	2830	71.29	25.19	3774	29.06	7.7
7	3774	114.21	30.26	2830	46.66	16.49	3774	16.57	4.39
8	4717	61.47	13.03	3774	43.09	11.42	5660	45.4	8.02
9	4717	42.42	8.99	3774	99.26	26.3	5660	59.04	10.43
10	5660	127.89	22.59	5660	32.68	5.77	7547	29.75	3.94

(b) Biomass-dependent faunal-mediated O_2 uptake

Linear ANCOVA (Analysis of covariance) was used to test for differences in faunal-mediated O_2 uptake among habitat types when controlled for biomass. Therefore, we tested if a shared relationship between biomass and faunal-mediated O_2 uptake can be

used to scale faunal mediated O_2 uptake to each of three different habitat types across the biomass range of *H. diversicolor*. Biomass of *H. diversicolor* was normalized via log transformation in order to meet linear ANCOVA assumptions of normality and homogeneity of variance, additionally ANCOVA-specific assumptions have been met. The level for statistical significance (α) was set at 0.05 in all analysis. The amount of faunal-mediated O_2 uptake (Y) performed by a homogeneous population are expressed as a combination of power functions of total biomass (W):

$$Y=cW^d \qquad (1)$$

where c is the quantified coefficient and d is the biomass-dependent scaling exponent.

Step 2: Extrapolation over quantile regression models: a mixed modelling approach with two temporal scenarios 1955/2010

Species distribution modelling (SDM) can be used to simulate change in ecosystem functioning (faunal-mediated O_2 uptake in the present case) under different environmental scenarios (e.g. Cozzoli et al., 2014; Gjoni et al., 2017; Queirós et al., 2015; Wrede et al., 2018). Here, the SDM couples abiotic descriptions of estuarine hydrology/morphology with a biotic model component describing the ecology of the target organism *H. diversicolor*. To compare the most recent changes of anthropogenic modification, the predictions of faunal-mediated O_2 uptake and species distribution maps were generated for two scenarios: (i) pre-1955 (herein '1955'), representing an anthropogenically unmodified estuarine environment, and (ii) post-2010 (herein '2010') following major infrastructural works in the Scheldt estuary. Extensive monitoring programmes of macrobenthic fauna have been executed over the past years in the Western Scheldt. Despite the large effort, field observations are intrinsically not sufficient to exhaustively and quantitatively reconstruct the changes undergone the Western Scheldt benthic ecosystem during recent decades. To bridge this gap, two quantile

regression models (upper quantile and full quantile models) were sequentially used to predict the temporal variation of species distribution, for the different purposes of estimating the maximal and realistic numerical distribution of *H. diversicolor*. More information on the quantile regression model fitting can be found in Cozzoli et al. (2017, 2014, 2013).

First, 1955 and 2010 hydrodynamic model scenarios of the Western Scheldt were used following Cozzoli et al. (2017). Briefly, two detailed bathymetric maps from 1955 and 2010 were used to produce simulated hydrodynamic scenarios using 2Dh TELEMAC of the Western Scheldt with the spatial resolution up to 20 m on the tidal shoals (Smolders et al., 2013). The hydrodynamic model is able to predict, for any sampling point in the estuary, yearly averages of maximal tidal current velocity (m s^{-1}), average salinity, salinity range (the diel variation in salinity) and submersion time (% of time for which the site is submerged during a tidal cycle). These variables are known to be among the most important in determining the benthos distribution in estuaries, including the distribution of *H. diversicolor* (Ysebaert et al., 2005, 1998). More information on the hydrodynamic model can be found in Smolders et al. (2013).

In a second step, the 2010 hydrodynamic scenario was used to extract hydrodynamic variables values for 3051 benthic samples collected between 2006 and 2011 in the Western Scheldt from the NIOZ-Yerseke Monitor Taskforce and recorded in the BIS dataset. Quantile regression was used to model the distribution of *H. diversicolor* in 2010 with respect to the aforementioned hydrodynamic variables. Quantile regression can fit any desired quantile of a response variable distribution to an independent variable and provide extra information about the probability of a realized density and biomass under given conditions (Bassett and Koenker, 1982; Koenker and Hallock, 2001). This allows to represent the heterogeneity in the response variable that commonly occurs in ecological

processes when only a subset of the relevant variables that may be limiting have actually been measured and incorporated into the models (Cade and Noon, 2003). The use of an upper quantile regression model (*i.e.* 0.95 quantile) estimates the maximum possible biomass achievable for a given combination of explanatory variables. Considering that unknown subsidiary factors and sampling stochasticity are likely to have a negative effect on the observed abundances or biomasses, models of the upper quantile can be used to produce a description of the niche potential and habitat suitability focused on the effect of the known explanatory variables (Anderson et al., 2008; Blackburn et al., 2006; Cade and Noon, 2003; Cozzoli et al., 2013). Habitat suitability is preferred as a reference parameter for spatial management strategies (Degraer et al., 2008), therefore the spatial mapping of faunal-mediated O_2 uptake based on maximal biomass is important for management purposes. However, upper quantile models provide good estimation of macrobenthic habitat suitability at the price of overestimating the realized abundances or biomasses by *ca.* 5 – 10 times (Cozzoli et al., 2014). Alternatively, estimating the full quantile range (*i.e.* quantiles 0.01 – 0.99) allows to predict the whole cumulative distribution of response values that could be realized for a given combination of known explanatory variables under the realistic influence of subsidiary factors. Therefore, full quantile range regression models allows to estimate a realistic numeric distribution of abundances or biomass over a given extensive environmental scenario (Cozzoli et al., 2014). In all regression analyses, submersion time and salinity range were arcsin and log transformed respectively, and biomass was log (x+1) transformed to meet normality assumptions. The maximal model was simplified using a bi-directional elimination stepwise procedure based on AIC comparison stepwise reduction procedure. Summary of quantile regression models in predicting maximal and realized biomass of *H. diversicolor* can be found in supplementary material Table 5.1. Please refer to

supplementary material Figure 5.3 for the model diagram of species distribution modelling.

As third step, the results of the quantile distribution model of *H. diversicolor* biomass were extrapolated on a whole basin scale for both the 2010 and the 1955 hydrodynamic scenarios. To provide a spatial comparison of maximal faunal-mediated O_2 uptake and the numeric distribution of the realized bioturbation effects on O_2 consumption at the estuarine landscape scale, both the upper and full quantile models were used. Following model integration, the predicted biomass of *H. diversicolor* from the upper quantile model and full quantile model were limited to the maximal and minimal observed values to have more conservative estimates of the species' effect on sediment metabolism.

Finally, Eq. 1 was applied to convert the estimated *H. diversicolor* biomass in potential (upper quantile model) and realized (full quantile range model) effect on O_2 consumption. The modelled scenarios for the years 1955 and 2010 were compared to investigate changes in *H. diversicolor*-mediated O_2 consumption in relation to the recent anthropogenic modifications of the estuary.

5.3 Results

5.3.1 Density-dependent effect on sediment O_2 consumption and upscaling result

There was a significant increase in faunal-mediated O_2 uptake with an increase in *H. diversicolor* biomass ($F_{1,26} = 119.6$, $p < 0.001$; Table 5.2; Figure 5.2); a response unaffected by habitat type ($F_{2,26} = 3.12$, $p = 0.06$; Table 5.2). The presence of *H. diversicolor* generated the faunal-mediated O_2 consumption up to 170.12 mmol m^{-2} d^{-1} at a high density of 5660 ind m^{-2} (Table 5.2). Faunal-mediated O_2 uptake was best explained by a weak power relationship; for every g·m^{-2} of AFDW biomass, O_2 uptake increased to the power 0.71.

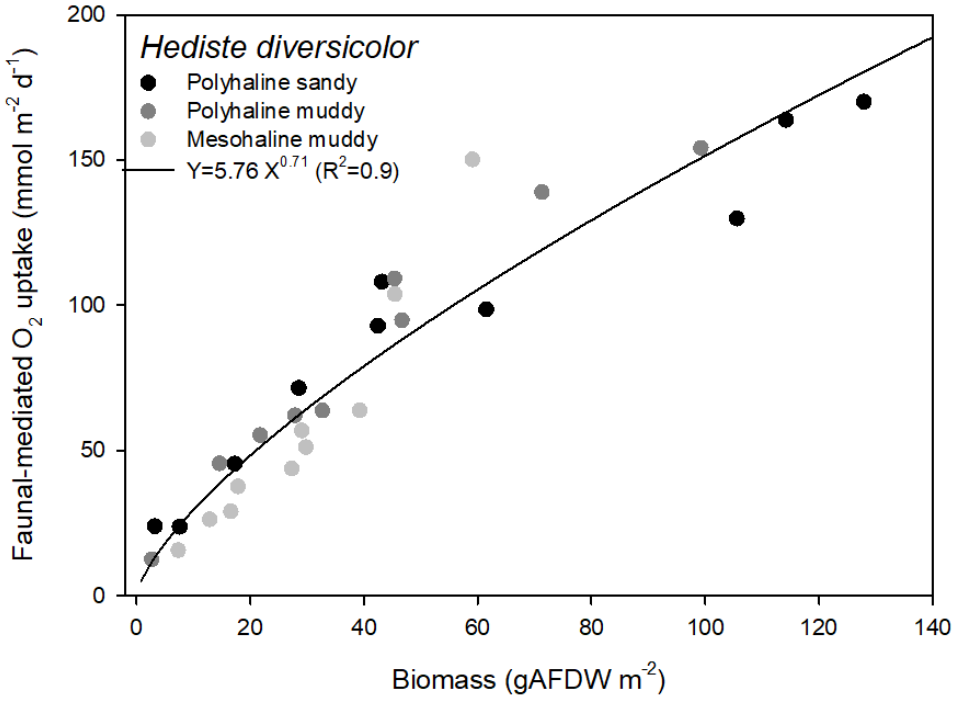


Figure 5.2: Faunal-mediated O_2 uptake (mmol m^{-2} d^{-1}) measured in each microcosm with the addition of *H. diversicolor* and the biomass-scaling functions ($p<0.05$).

Table 5.2: ANCOVA results. Faunal-mediated O_2 uptake is the dependent variable, Habitat is the factor, and biomass is the covariate.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	53287.82	3	17762.61	45.53	<0.001
Intercept	8890.45	1	8890.45	22.79	<0.001
Biomass	46653.97	1	46653.97	119.58	<0.001
Habitat	2436.46	2	1218.23	3.12	0.06
Error	10143.77	26	390.15		
Total	245750.61	30			
Corrected Total	63431.60	29			

a. R^2= 0.84 (Adjusted R^2 = 0.82)

5.3.2 Landscape effect of *H. diversicolor* on O_2 fluxes

The analysis of Western Scheldt abiotic scenarios showed different trends over the time span and region-specific patterns of environmental predictors. In the 1955 scenario, the current velocities of the Western Scheldt fell in the range 0-1.6 m s^{-1} whereas the maximum velocity increased to 2.14 m s^{-1} in 2010 (Table 5.3) and the increment mainly occurred in the subtidal regions in the channel and marine mouth region, not on the intertidal flats (Figure 5.3a, 5.3b). The mean value of maximum current velocity of the whole Western Scheldt significantly increased from 0.73 m s^{-1} in 1955 to 1.06 m s^{-1} in 2010 ($t=691.96$; $p<0.05$). Increased spatial extent of intertidal flats were submerged in the water in 1955 and the elevation of intertidal areas with reduced submersion time was revealed in 2010 (Figure 5.3g, 5.3h).

Salinity variation on daily basis was slightly widened to 0-14.4 in 2010 compared with 0-13.11 in 1955, with the mean salinity range 2.57 and 3.2 in 1955 and 2010 respectively. A t-test showed a significant rise in daily variation in salinity ($t=191.25$; $p<0.05$), particularly at the inner side of a bend at the northern part of Hooge Platen (Symbol A; Figure 5.3e, 5.3f). The area close to the intertidal flat Verdronken Land van Saeftinghe in the mesohaline region was characterized by maximum daily salinity variation up to ~14 in 1955, and in 2010 the area with high salinity variation expanded over the bend, approaching several mudflats in the surroundings (Symbol B; Figure 5.3e, 5.3f). In spite of the morphological variations, the mean salinity has been relatively stable (Table 5.3 Figure 5.3a-5.3h) generating the salinity gradients from polyhaline to mesohaline.

Table 5.3: Range of abiotic variables used in the models in 1955 and 2010

	Maximum current velocity ($m.s^{-1}$)		Mean salinity		Salinity range		Submersion time (%)	
Year	1955	2010	1955	2010	1955	2010	1955	2010
Minimum	0	0	2.45	2	0	0	0.05	0.05
Maximum	1.6	2.14	35.85	35	13.11	14.4	1	1
Mean	0.73	1.06	27.5	28.1	2.57	3.2	0.91	0.92

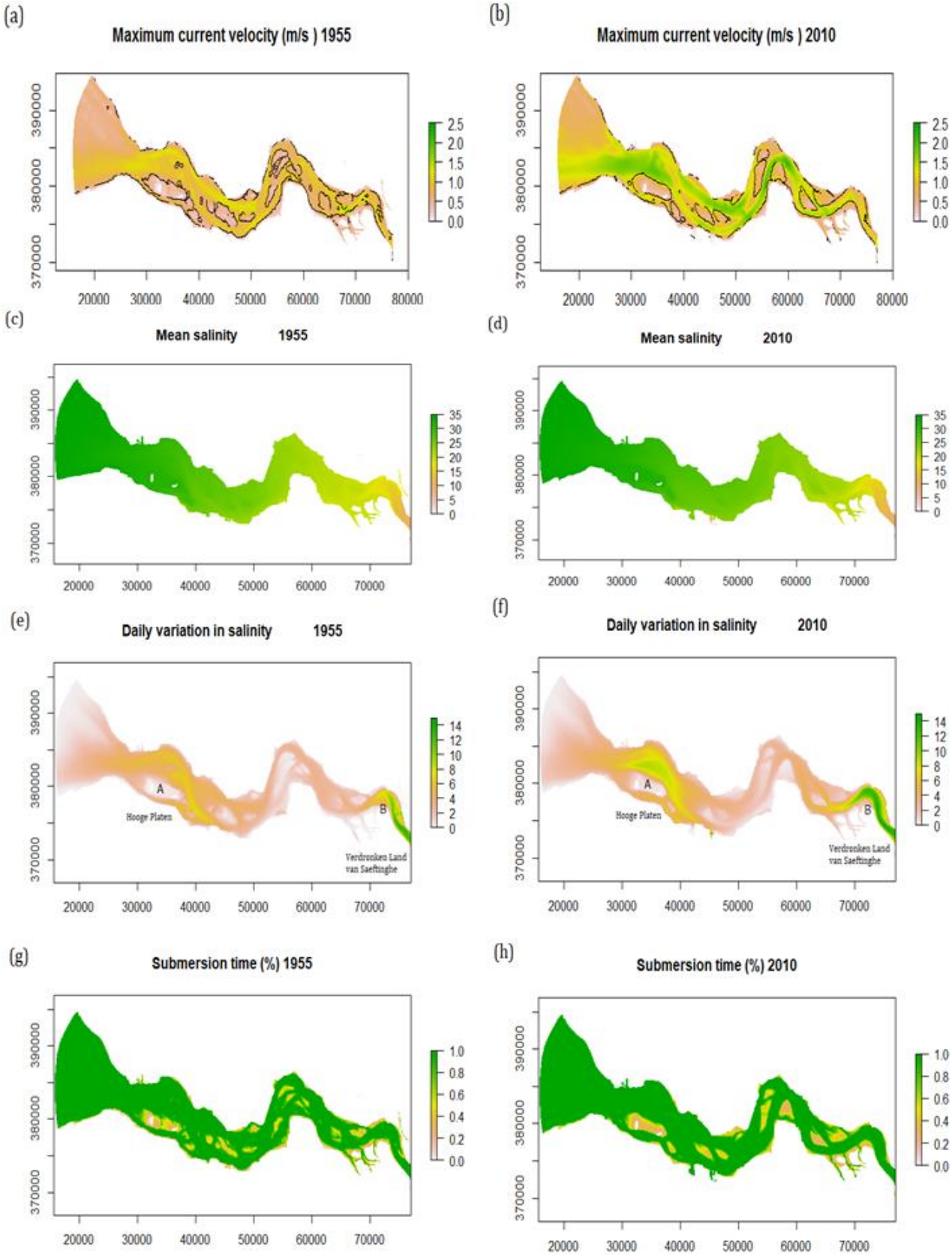


Figure 5.3: Spatial mapping of abiotic variables included in the model to predict the distribution of *H. diversicolor*: (a) maximum current velocity 1955, (b) maximum current velocity 2010, (c) mean salinity 1955, (d) mean salinity 2010, (e) daily variation in salinity 1955, (f) daily variation in salinity 2010, (g)

submersion time 1955, (h) submersion time 2010. Symbols in (e) and (f) are tidal flats in the Western Scheldt: (A) Hooge Platen, (B) Verdronken Land van Saeftinghe

Intertidal habitats were predicted as the most suitable habitat for *H. diversicolor* in the Scheldt estuary, and in particular, muddy intertidal environments (Figure 5.4a, 5.4b; Supplementary material Figure 5.1; upper quantile model). The potential faunal-mediated O_2 uptake was lower on the exposed edges of tidal flats compared with the inner part (Figure 5.4c); the comparison between 1955 and 2010 scenarios showed a net increase in the inner part of the tidal flat and a decrease at the edges. Maximal faunal-mediated O_2 uptake was 153.4 mmol m^{-2} d^{-1}, and the predictions for the whole Western Scheldt suggested a maximal total biomass of 4407.12 kg and 2915.03 kg, and O_2 consumption of 8772 and 6201 mol d^{-1} for 1955 and 2010 respectively (Table 5.4) representing a reduction of 34% and 29% in these metrics over this time period. The tidal flats along the channel at the southern part of the estuary, especially the bend close to Terneuzen (symbol C; Figure 5.4c) exhibits considerable spatial variation in the enhanced O_2 uptake; the mediated O_2 consumption was higher in the higher part of the tidal flats in 1955 up to ~153.4 mmol m^{-2} d^{-1} (Figure 5.4d), however the influenced region shrunk in 2010 (Figure 5.4a, 5.4b).

The full quantile model was used to predict the realized distribution and biomass of *H. diversicolor* (Supplementary material Figure 5.2) and landscape quantification of faunal-mediated O_2 uptake in 1955 and 2010. This revealed a decline in total biomass of *H. diversicolor* of 34% (from 3044.71 kg to 1040.03 kg; Table 5.4), which corresponded with a 39.7% reduction in the faunal-mediated O_2 consumption in the whole estuary from 5401.31 to 2145.93 mol d^{-1} (Table 5.4; Figure 5.5d). Greatest reductions were predicted for the southern river bank (Figure 5.5c), with many areas experiencing a greatly reduced

or total loss of realized faunal-mediated O_2 uptake in sediment metabolism, in part driven by a reduction in biomass of *H. diversicolor* along the estuarine margins (Table 5.4).

Table 5.4: The maximal and realized O_2 uptake (mol d^{-1}) enhanced by *H. diversicolor* estimated from upper quantile and full quantile models (sum only takes intertidal area into account, categorized by areas with submersion time<0.99).

	Upper quantile scenario of biomass			Full quantile scenario of biomass		
year	sum (kg)	max (g m^{-2})	min (g m^{-2})	sum (kg)	max (g m^{-2})	min (g m^{-2})
1955	4407.1	101.8	0	3044.7	101.8	0
2010	2915			1040		
	Maximal faunal-mediated O_2 uptake			Realized faunal-mediated O_2 uptake		
year	sum (mol d^{-1})	max (mmol m^{-2} d^{-1})	min (mmol m^{-2} d^{-1})	sum (mol d^{-1})	max (mmol m^{-2} d^{-1})	min (mmol m^{-2} d^{-1})
1955	8772	153.4	0	5401.3	153.4	0
2010	6200.7			2145.9		

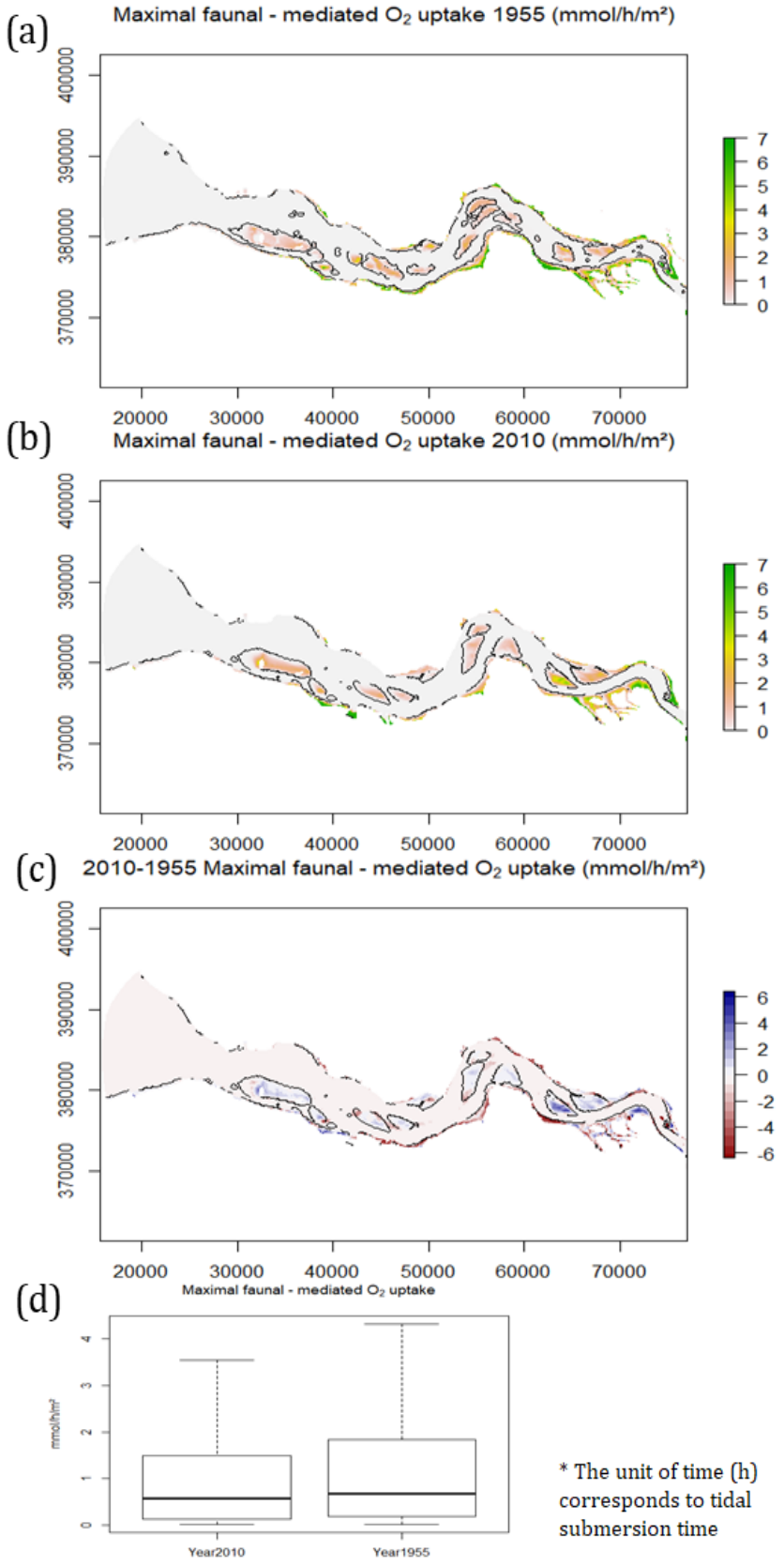


Figure 5.4: Maximal faunal-mediated O_2 uptake (mmol m^{-2} h^{-1}) during tidal submersion in the Western Scheldt in (a) 1955, (b) 2010, (c) Difference over the time span $\Delta_{2010\text{-}1955}$, and (d) Temporal comparison (1955 vs 2010) of the faunal-mediated O_2 uptake estimated from the upper quantile distribution (potential

niche) of *H. diversicolor* (Quantile regression, tau=0.95). Symbol (C) showed the location of the drastic spatial variation in O_2 uptake.

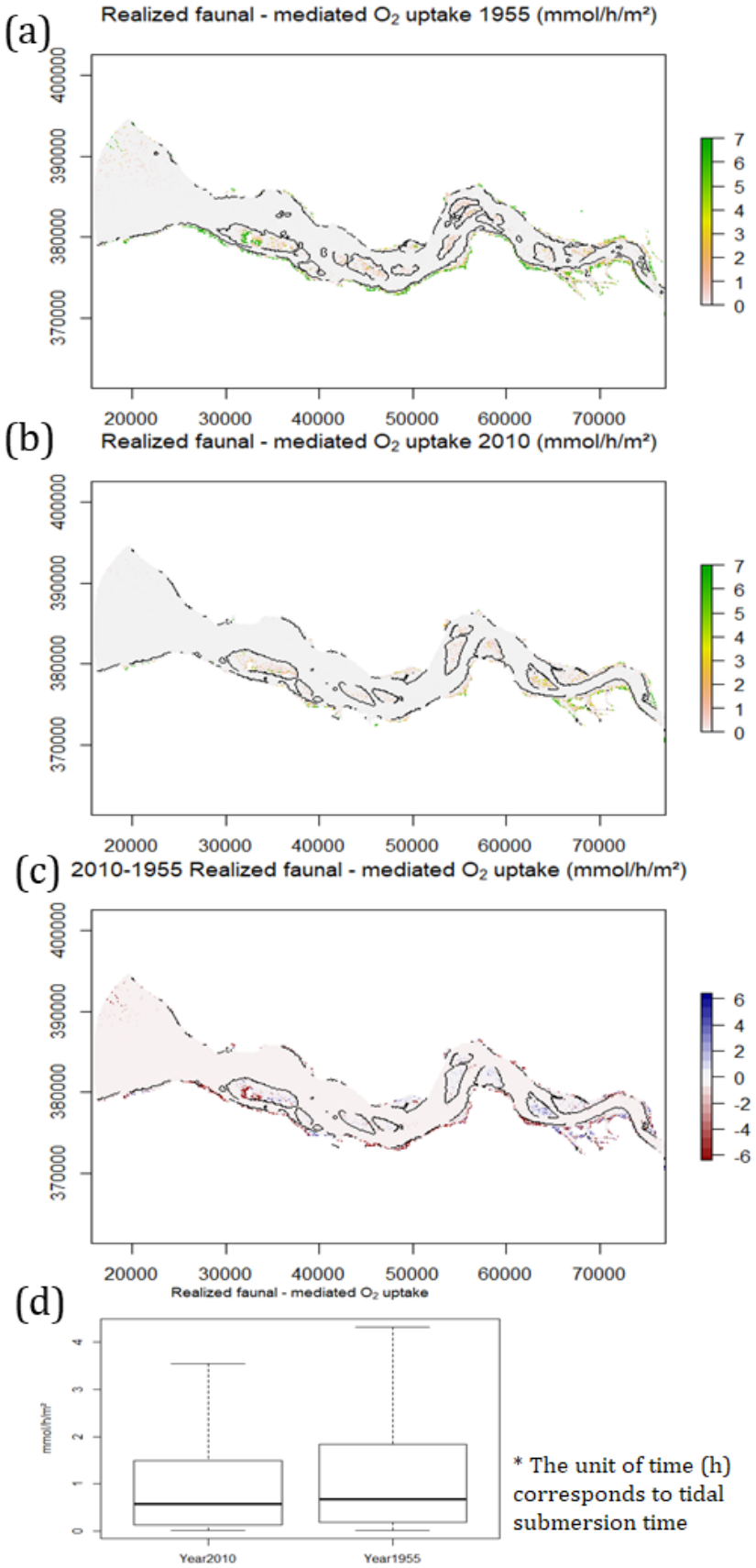


Figure 5.5: Realized faunal-mediated O_2 uptake (mmol m^{-2} h^{-1}) during tidal submersion in the Western Scheldt in (a) 1955, (b) 2010 and (c) Difference over the time span $\Delta_{2010\text{-}1955}$, and (d) Temporal comparison (1955 vs 2010) of the realized faunal-mediated O_2 uptake estimated from full quantile distribution of *H. diversicolor* distribution (Quantile regression, tau=0.01-0.99)

5.4 Discussion

There is now more incentive than ever for the development and introduction of environmental monitoring and management strategies that alleviate anthropogenic pressure from coastal ecosystems in order to maintain aquatic ecosystem health (Halpern et al., 2008; Knights et al., 2015; Vörösmarty et al., 2010). Yet, incentive requires evidence of impact to underpin decision-making (Knights et al., 2014). Here, we show how the use of species distribution modelling (SDM) that builds on a robust understanding of ecosystem functioning allows the environmental (e.g. geomorphological) effects of anthropogenic and/or natural change of an estuarine system to be predicted with confidence. We did this by first quantifying the ecosystem functioning contribution of an important macrobenthic bioturbator, *Hediste diversicolor*, using mesocosm experiments, which was then upscaled using SDM to estimate whole ecosystem functioning underpinning environmental policy and management decision-making. Using the Western Scheldt estuary as a case study, this strategy bridges small-scale experimental studies with the broad-scale mapping needs of society and managers forming a predictive framework that informs policy makers and conservation practitioners for sustainable management of estuaries. This approach could be used to predict faunal-mediated O_2 consumption over large benthic landscapes at high spatial resolution caused by changes in population density of benthic bioturbators, further estimate ecosystem-scale changes for managing suitability over large temporal scale.

5.4.1 Landscape-scale effects of *H. diversicolor* on oxygen dynamics (1955 vs 2010)

Application of different temporal scenarios, which project changes in abiotic environment and species distribution over time, provides insights into change in ecosystem functioning. In this study, quantile regression modelling was used to provide a landscape-scale overview of spatio-temporal change in ecosystem functioning and service provision across the heterogeneous environmental conditions of the Western Scheldt. By fitting different quantiles (i.e. tau=0.95 and 0.01-0.99), two species distribution scenarios were developed to describe the organisms' response to different environmental constraints. The upper quantile scenario - which predicts maximum biomass - indicated a marked decrease in availability of suitable habitat for *H. diversicolor* across the Western Scheldt between 1955 and 2010 (Supplementary material Figure 5.1). In contrast, predictions of sediment oxygenation by *H. diversicolor* in intertidal flats showed contrasting trends depending on location (Fig. 5.4c). Inside the estuary, there was an increase in oxygenation (Blue colour; Figure 5.4c); whilst along the estuarine margins, there was a general decline of approximately ~150 mmol m^{-2} d^{-1} (Red colour; Figure 5.4c). Overlaying human activities suggest that the loss and/or reduction in *H. diversicolor* habitat along the river channel closely coincides with dredging and spoil dumping activities (Figure 5.6).

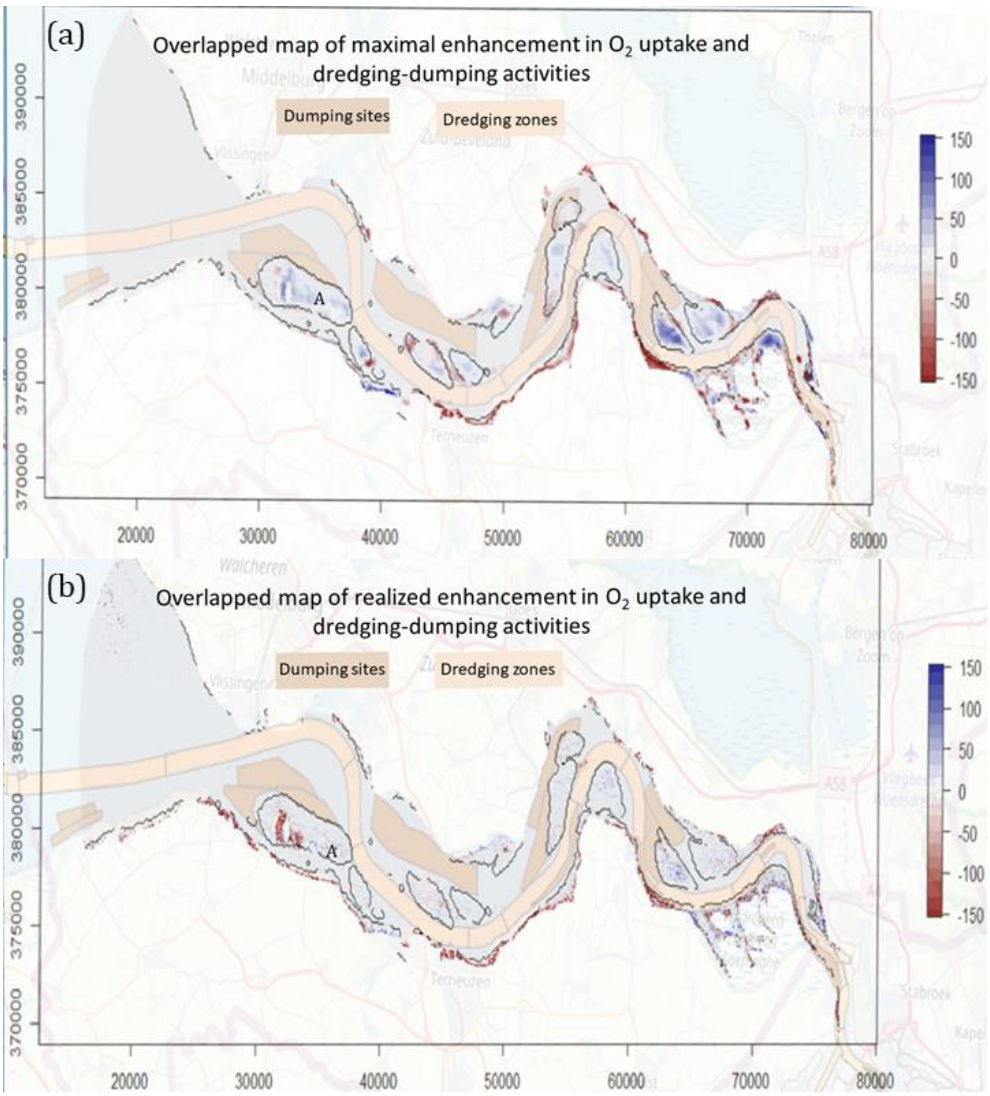


Figure 5.6: Overlapped maps of temporal variation (2010-1955) in (a) maximal and (b) realized faunal-mediated O_2 uptake (in mmol m^{-2} d^{-1}) of tidal submersion and dredging-dumping activities summarized until 2010 (*http://www.scheldemonitor.be*). Symbol A is the tidal flat Hooge Platen, where the adverse effects were predicted by upper quantile and full quantile scenarios.

The full quantile model predicts the effect of environmental change on realized biomass which has significant effect on ecosystem functioning and is thus a valuable tool for ecological management (Cozzoli et al., 2014). Here, incorporating both spatial extent of suitable habitat and biomass predicted a 60% decline in oxygenation function provided by *H. diversicolor* (2145.9 and 5401.3 mol d^{-1}; Table 5.4) between 1955 and 2010. These estimates were 34.6% and 61.6% smaller than the 1955 and 2101 estimates generated using the upper quantile model respectively (6200.7 and 8772 mol d^{-1}; Table 5.4) and highlights the importance of coupling biological responses to environmental conditions (Cozzoli et al., 2014) in species distribution models. Therefore, the biotic response incorporates the influence from other unmeasured environmental variables, the variation of which might have negative effects on the functioning generated by *H. diversicolor*. An increase of oxygenation was found in intertidal zones inside the channel and a decrease occurred to the marginal zones of the estuary, whilst in some specific sites the adverse pattern was revealed (Figure 5.5c). For example, the western edge of the tidal flat Hooge Platen showed reduced oxygenation in 2010 (Symbol A; Figure 5.5c). The elevation of the mudflat (Figure 5.3g, 5.3h) and the reported shoal margin failure (van Dijk et al., 2019) might cause the adverse biotic response of *H. diversicolor* in Hooge Platen (Symbol A; Figure 5.5c).

5.4.2 Impacts of direct and indirect human-induced disturbance on *H. diversicolor*

The Western Scheldt has been subject to constant change in natural processes and in the intensity of anthropogenic activities between 1955 and 2010. Anthropogenic activities have substantially modified the depth, direction, and velocity of water flows, contributing to an increase in the height and steepness of tidal flats over the past decades (de Vet et al., 2017). The increase in elevation of intertidal flats since 1955 (Figure 5.3) has led to

the modification of *H. diversicolor* habitat, limiting its presence due to reductions in submersion time as well as a greater risk of potential collapses of tidal flats and shoals. Since the shoals and tidal flats inside the estuary are not protected against erosion and the channel bed is covered by fine sediments with a median grain size between 200 and 300μm and 10–20% of the intertidal areas is dominantly covered by mud (Braat et al., 2017), the tidal flats would be directly influenced by the changes in hydrodynamics leading to vulnerability to the risk of channel bank collapse. There is considerable sediment loss brought by shoal margin collapses such that several millions m^3 of sediment collapsed per event (van Dijk et al., 2018), which even approached the annually dredged volumes (Jeuken and Wang, 2010), directly leading to the loss of natural habitats for *H. diversicolor*.

In the Western Scheldt, dredging increased from less than 0.5 million m^3yr^{-1} before 1950 to about 7–10 million m^3yr^{-1} at present (Hibma et al., 2008). Studies indicate that dredging causes a reduction in the abundance, species diversity, and biomass of the benthic community (for review see Newell et al., 1998). In the physical context, modification of channel morphology as a result of dredging operations to provide access to the Port of Antwerp have led to an increase in maximum tidal current velocity (Figure 5.3a, 5.3b). Due to the close association among the environmental factors, the impact of higher-velocity hydrodynamics may further lead to the changes in mixing dynamics between estuarine and marine regions. As revealed in the 2010 scenario, the salt front of seawater intrusion has been shifting upstream and altering the tidal environment in the estuary compared with the 1955 scenario (Symbol A; Figure 5.3e, 5.3f), thus the potential change of the composition of macrobenthic community is expected. Even though *H. diversicolor* is tolerant to great variation of salinity (Smith, 1956), its response (i.e.

biomass) to the changes of spatial distribution of other benthic species that are sensitive to salinity change would be expected.

The alteration in sediment via dredging and dumping operations may not only affect the physical appearance and hydrodynamics of the ecosystem (Monge-Ganuzas et al., 2013), but also affect the estuarine sediment dynamics by greatly increasing the suspended sediment concentration in the water column (van Maren et al., 2015). In the biogeochemical context, dredging practices can cause additional complications for the benthic biogeochemistry: buried organic matter can resurface after the topmost sediment layers are removed, thus leading to the releasing of previously 'lost' nutrients and possible contaminants from the seafloor (Eggleton and Thomas, 2004; Lohrer and Wetz, 2003), causing additional complications for the benthic biogeochemistry (Essink, 1998). Mestdagh et al. (2018) observed that piston-pumping of polychaetes intensified under lower sediment deposition, whilst ceased under high deposition due to sediment ingestion, corresponding to natural and dredging scenarios. The increased turbidity induced by dredging may lead to significant impact on the biogeochemistry through changes in behaviour of macrobenthic bioturbators (i.e. particle reworking and bio-irrigation).

Dumping of dredged sediments lead to enhanced sediment deposition at dump sites, and this would not only directly affect growth and survival of macrobenthos, but also influence primary production by phytoplankton and performance of visual predators (e.g. fish, birds) (Essink, 1999). Mortality of the organisms is expected to occur due to the burial by dumped sediments and enhanced suspended matter would impair the growth of some species (e.g. filter-feeding bivalves) (Essink, 1999). Besides, the morphological impact of dumping activities in the Western Scheldt with a weaker flood dominance was modelled by Hibma et al. (2008), the dumped sediment accumulates more likely around

the dumping location and possible channel degeneration in the Western Scheldt evolving from shoaling around the dumping area without migration of the shoal is expected (Figure 5.3).

Considering the distribution of benthic species is constrained by an array of environmental variables (i.e. salinity, granulometric properties of the sediments), the biological consequences of sediment changes can be assumed extensive in estuarine ecosystems. In this study, the decrease of total biomass of *H. diversicolor* in the estuary from 1955 to 2010 has contributed to the decline of total faunal-mediated O_2 uptake at the estuarine landscape scale (Table 5.4). Increases in human-induced disturbance (e.g. dredging and dumping), has altered the functioning (e.g. faunal-mediated O_2 uptake) of *H. diversicolor* as a result of: (1) destruction of natural habitats and burial by dumped sediments, leading to the decline of biomass thus less faunal-mediated O_2 consumption, and (2) the indirect effects of abiotic alterations (e.g. enhanced hydrodynamics, seawater intrusion and higher loading of suspended sediments) and associated variation in biotic community (e.g. phytoplankton, predator and benthic community), which influence the mediation by *H. diversicolor* in benthic processes (e.g. bioturbation) and biogeochemical processes in sediment. Besides, bioturbation effect (e.g. modification of sediment properties) can also facilitate or prevent the settlement of other species (Commito et al., 1995). Thus *H. diversicolor* might generate themselves feedback dynamics (Jones et al. 1997), in this case the bioturbation effect by *H. diversicolor* on other species affect the determinants of its own distribution and behaviours.

Human-induced disturbance in the Western Scheldt affected mediated O_2 uptake by *H. diversicolor* both directly and indirectly, leading to the negative relationship between human alteration (e.g. dredging) and faunal-mediated O_2 uptake; whilst numerous indirect effects are likely to reverberate throughout the ecosystem (e.g. relative exposure,

wave action, permeability, porosity or oxygen content) (Gray, 1974). Though the drastic decrease of biomass of *H. diversicolor* in the whole Western Scheldt further caused the decline in the faunal-mediated O_2 consumption; however spatially, in some intertidal zone (e.g. Hooge Platen) along the river channel the increase of mediated O_2 uptake was indicated in realized scenario by comparison between 2010 and 1955.

5.4.3 Model improvements

Differences between the upper and full quantile models suggest sensitivity to the inclusion/exclusion of potential explanatory variables. The models were constructed using the outcomes of mesocosm experiments described in **Chapters 2-4**, and here I suggest some improvements that could further refine these predictions:

Here, there was limited consideration of environmental spatio-temporal heterogeneity and mobility between closely-spaced patches (Levinton and Kelaher, 2004); processes that to date have scarcely been accounted for in modelling bioturbation and ecosystem functioning (Delmotte et al., 2008; but see Cuddlington and Hastings 2004), but could induce patchy bioturbation and impact sediment biogeochemistry estimates. Here, the scale of patchiness was unknown, but a valuable next step may be to better (spatially) resolve the scales of patchiness in species' distribution which may increase the accuracy of bioturbation estimates. Temporal heterogeneity - such as that induced by hydrodynamic conditions during extreme events - may temporarily impose strong structuring forces that markedly alter the dynamics of the ecosystem (e.g. Koppel et al. 2005; Leonard and Luther 1995), and are predicted to increase and intensify in the future (Ghedini et al., 2015; Wernberg et al., 2013). Thus, it is worth capturing this temporal variability and used the approach presented in this study (e.g. comparison of 1955 vs 2010) as a framework.

Given the differences between the upper and full quantile model estimates, it is suggested that the effect of incorporating abiotic and biotic changes within models should be further considered. Anthropogenic pressures caused by coastal human activity lead to the environmental consequences such as hydrodynamics and habitat configuration. Climate change is expected not only to change the physical system (increase storminess and sea level rise) but also the biological processes (change in zonation of biota, invasions of exotic species and increase of water temperature) (Parmesan, 2006). These abiotic and biotic changes would profoundly impact ecosystem properties and patterns (see thesis **Chapter 2**-**4**). Thus, future scenarios related to anthropogenic climate changes are recommendable to be incorporated, to secure future delivery of ecosystem functions/services. It is certainly not feasible to test the response of species and ecosystems to all the anthropogenic climate drivers including local variability, several specific aspects are suggested below to develop future scenarios with a focus on key drivers and crucial processes: (1) The thresholds of temperature. Variation in temperature fundamentally affects biological processes since animal metabolism is temperature-dependent. Though organisms are able to adapt to a range of temperatures, once the temperature increases exceeding the threshold values, reduction in fitness, increase in mortality, decline in population are expected (Hochachka and Somero, 2002). In this regard, increasing temperatures in mesocosm experiments to explore the warming effect on biological processes (life history traits, population growth, species distribution) and ecosystem functions (i.e. biogeochemical fluxes) would be able to identify the specific thresholds, contributing to the understanding of potential ecological shifts with spatial and temporal details. Other symptoms of global change such as acidification, hypoxia, and sea level rise can also be manipulated in the laboratory, to provide a mechanistic understanding of each driver. (2) These drivers of anthropogenic climate change add to the complication of ecological systems, potentially triggering the

feedback loops and domino effects (Hoegh-Guldberg and Bruno, 2010), therefore future scenarios incorporating multi-drivers could be useful to predict the potential consequence,

Overall, the negative anthropogenic impact on marine species and ecosystems is certain and rapid and dramatic ecological shifts are occurring with clear socio-economic consequences. However, there are strategies to move forward to reduce growing risks of pushing current ecosystems into a more dangerous state. Key species and processes are a key to project future impacts. To maintain the function of estuarine ecosystems and to secure the services they deliver under future anthropogenic influence, we therefore need to ensure these crucial components (i.e. key species and processes) are understood, modelled, monitored and prioritized in management frameworks.

5.5 Conclusion

The benthos-ecosystem functioning relationship was approximated in this study by integrating biomass-dependent faunal-mediated O_2 uptake and species distribution models. The maximum faunal-mediated O_2 uptake by *H. diversicolor* indicated a reduction in ecosystem function provision of ~29%, whilst the realized faunal-mediated O_2 uptake indicated a reduction of ~60% between the same period of 1955 to 2010. The biomass of *H. diversicolor* and the bioturbation effect (e.g. bio-irrigation) were driven by the changes in channel morphology and biogeochemical properties of the sediments and linked to dredging-dumping practices. Environmental consequences of dredging-dumping operations (e.g. decrease in flow velocities, increase of sediment suspension, nutrients and morphological modification) have led to reduced biomass and mediated O_2 uptake by *H. diversicolor* between 1955 and 2010, therefore efforts to protect remaining habitats are needed. Loss of key bioturbators such as *H. diversicolor* will have consequences for

oxygenation, resulting in far-reaching impacts on ecosystem, such as a decrease in favourable niches for other infauna.

The approach applied in this study bridges small-scale experimental studies with the broad-scale mapping needs of society and managers forming a predictive framework that informs policy makers and conservation practitioners for sustainable management of estuaries. The proposed predictive framework is therefore expected to be generalized to quantify the bioturbation impact of other key species on landscape evolution and ecosystem functionality.

Acknowledgement

The authors greatly acknowledge Monitor taskforce (NIOZ-Yerseke) for providing access to benthic dataset and Sven Smolders for hydrodynamic modelling. We also thank the colleagues from UGent for their help in sampling campaigns. This work was funded by the MARES doctoral programme (2012-1720/001-001-EMJD) and EMBRC Belgium – FWO project GOH3817N.

Chapter 6 General discussion

6.1 Thesis aim

The aim of this thesis was to provide a comprehensive examination of the roles of macrofauna on estuarine biogeochemistry, by adopting a multidisciplinary field and laboratory-based approach and integration of species distribution models and biomass-dependent ecosystem functioning models (Figure 6.1). Efforts have been made to explore the species mechanisms in facilitating biogeochemical fluxes and the feasibility of utilizing metabolic scaling to quantify the contribution from key bioturbating fauna to sediment metabolism.

Given the key findings gained from the baseline survey in the Scheldt estuary (**Chapter 2-3**) followed by experiments to quantify the functioning of habitats with different infaunal densities under a range of environmental conditions (**Chapter 4**) allowed the role of bioturbators across a range of spatio-temporal scales to be determined. A case study was then conducted to assess change in functioning between 1955 and 2010 to reflect change between pre- and post-anthropogenic modification, and to give an estimate of the total value of the faunal-mediated O_2 consumption for the Western Scheldt (**Chapter 5**).

This thesis specifically addressed the following objectives:

- Explore the spatio-temporal variation patterns in sediment ecosystem processes (e.g. particle mixing and bio-irrigation) and quantify contributions from key macrofaunal species in bioturbation (**Chapter 2**)
- Identify the role of macrobenthos functional traits and spatio-temporal variability in biogeochemical process (**Chapter 3**)

- Investigate the effects of key benthic bioturbators' population density, habitat and species identity on intertidal sediment metabolism (**Chapter 4**)
- Spatial mapping of benthos-mediated ecosystem functioning at estuarine landscape level to assess ecosystem management (**Chapter 5**)

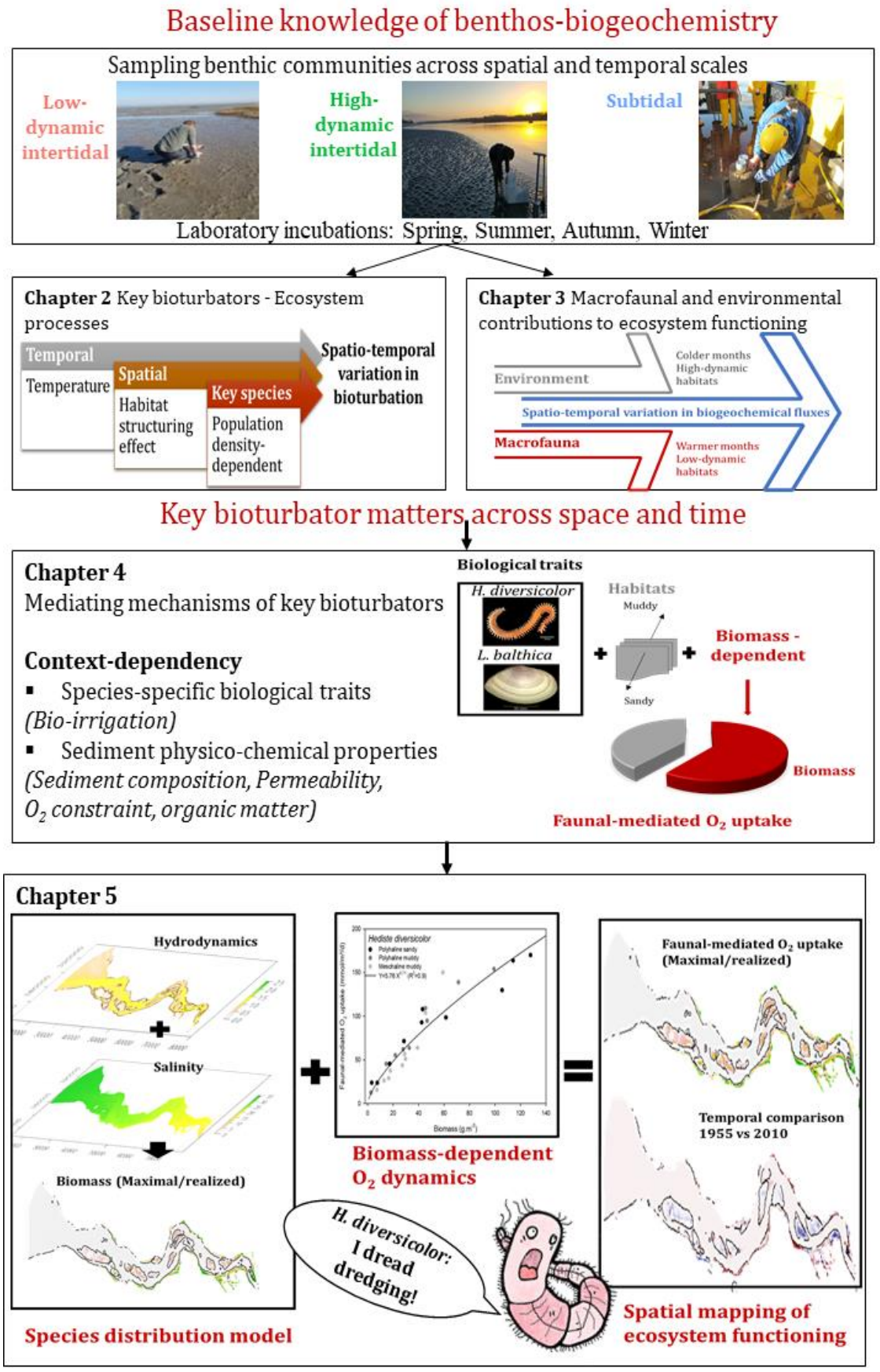


Figure 6.1: A schematic diagram presenting the overview of the structure and the key findings of the thesis.

6.2 Benthos contribution to ecosystem functioning across space and time

In marine ecosystems, consensus exists that functional traits mediate ecosystem processes and functions (Norling et al., 2007; Solan et al., 2004a; Wrede et al., 2018). This resulted in the application of trait-based indices of functional diversity, from which a mechanistic understanding of the biotic control on ecosystem functioning and service delivery is expected to be acquired (Gagic et al., 2015; Mokany et al., 2008; Petchey and Gaston, 2006). The good performance by the species-specific trait-based models has been confirmed in quantitatively predicting particle reworking (Queirós et al., 2015; Solan et al., 2004a) and bio-irrigation (Wrede et al., 2018). However, challenges remain in such general mechanistic framework due to the high variation in bioturbators' species distribution and functional behaviour, such that the contributory roles of species vary in time and across space (Godbold et al., 2011; Needham et al., 2011) . These approaches assume functional consistency and evaluate the mean performance, without considering variation in trait expression in evaluating the community performance (Violle et al., 2007). It has been advocated to consider the effects of environmental conditions on trait values used and adjust corresponding context-specific reworking and mobility trait scores when information about the alteration of species behaviour in response to external stimuli is known (Queirós et al., 2013). This is because some studies have shown that the summation of single species effects cannot accurately assess the community effect on biogeochemical cycling (Hale et al., 2014; Michaud et al., 2005), which underestimates the variation of the intraspecific traits and different effects within and between populations (McCain et al., 2016), and do not consider the role of biotic heterogeneity (e.g. predation, competition) (Alexander et al., 2015; McCain et al., 2016)

or change in environmental conditions (e.g. temperature, hydrodynamics, food resources; Mrowicki and O'Connor, 2015; Ouellette et al., 2004).

This thesis demonstrates that different benthic species contribute unevenly to both particle reworking and bio-irrigation processes (**Chapter 2**), with the habitat structuring effects on populations, density-dependent interactions with the habitat, and temperature-driven variability in macrobenthos activity and living position suggested to explain the observed spatio-temporal patterns. The PCA analysis based on a suite of experimentally derived and modelled variables related to particle reworking reveals that maximum penetration depth is also instrumental in explaining variability in particle reworking in space and time predicting particle reworking (**Chapter 2**).

The patterns of biogeochemical fluxes in the Scheldt estuary relate to the biotic regulation by the activities of macrobenthic functional groups (e.g. particle mixing and bio-irrigation), and the abiotic gradients (e.g. sediment organic content) along the estuary and between seasons (**Chapter 3**). Variation partitioning based on the seasonal observational data showed that macrofauna contributed most to sediment biogeochemistry in September and December and environmental conditions most in March and June. Furthermore, macrofaunal contribution to biogeochemistry was found to be higher in low-dynamic intertidal than in high-dynamic intertidal and subtidal habitats (**Chapter 3**). Though highly variable among seasons and habitats, biomass of key species is a better predictor in explaining the variance of measured ecosystem processes (i.e. particle reworking and bio-irrigation) in the seasonal survey in comparison to biodiversity attributes (**Chapter 2-3**) (Figure 6.2).

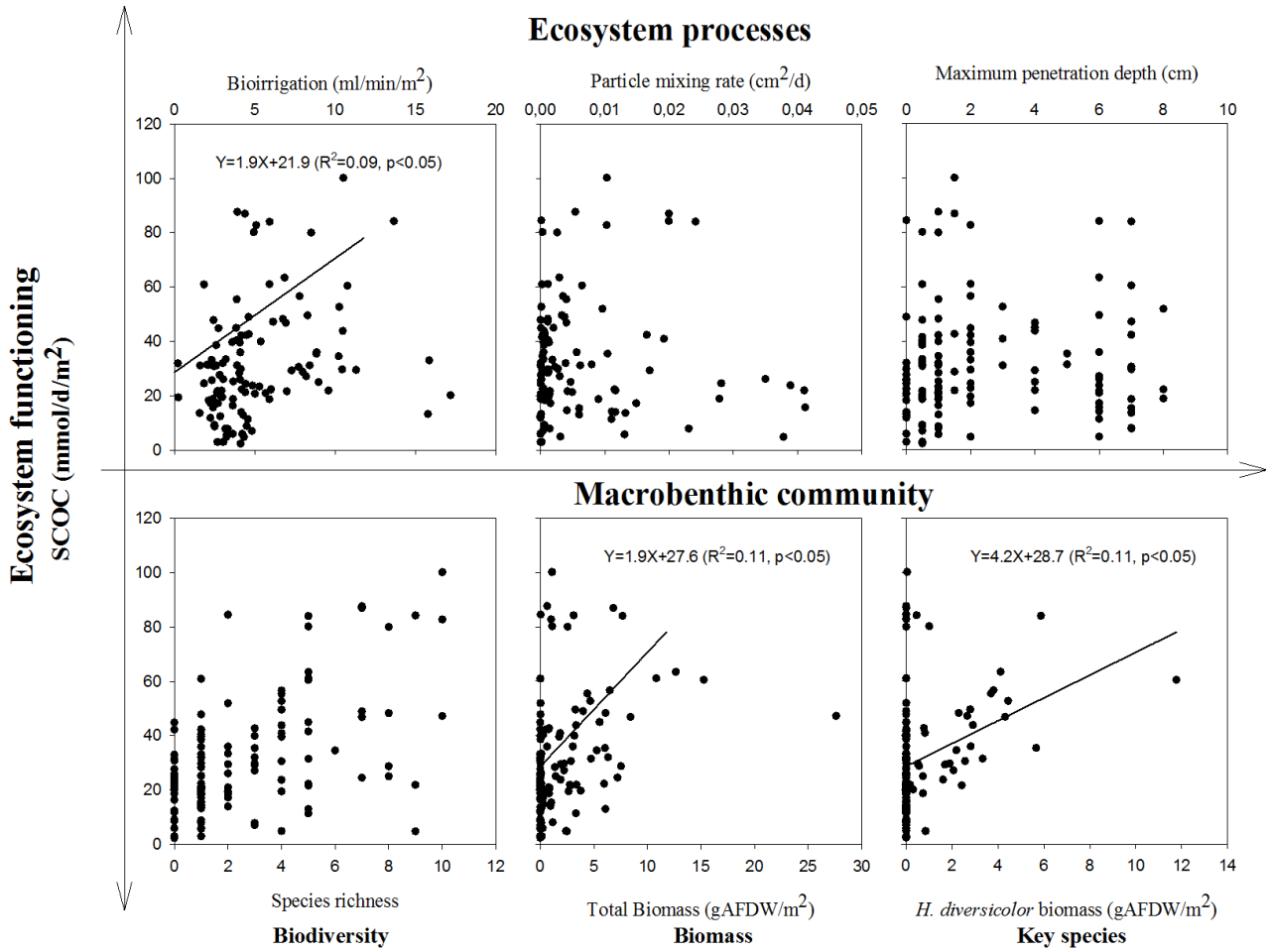


Figure 6.2: Relationships between benthic predictors and SCOC (Sediment community oxygen consumption) (**Chapter 2-3**). The x-axis represents benthic predictors: benthic community (i.e. biodiversity, total biomass, biomass of key species) and ecosystem processes (i.e. bio-irrigation, particle mixing rate, maximum penetration depth); y-axis represents SCOC measured in intact sediment cores collected from seasonal baseline survey in the intertidal area (2015-2016). Panels show how 6 benthic variables: (a) bio-irrigation (ml min m^{-2}), (b) particle reworking rate (cm^2 d^{-1}), (c) maximum penetration depth (cm), (d) species richness, (e) total biomass (gAFDW m^{-2}) and (f) *H. diversicolor* biomass, influence SCOC.

Some coherent sets of properties in ecological relationships emerge across scales, when the detailed observational data from intact sediment cores (baseline survey; **Chapter 2** and **Chapter 3**) and the data of single species response from manipulative experimentations (**Chapter 4**) are integrated. Firstly, the positive relationship between the biomass of key species *H. diversicolor* and total oxygen consumption across space and time scales can be observed (Figure 6.2), given the amount of environmental heterogeneity represented by the sampling sites and times in the baseline survey (**Chapter 2** and **Chapter 3**). Surprisingly, though the measured SCOC in baseline survey included the whole macrobenthic community, it is consistent with results from the density-dependent experiment schemes (**Chapter 4**) in monoculture of *H. diversicolor* and *L. balthica* (Figure 6.3). This is aligned with previous observations that the strongest bioturbators can superimpose the effects of the other species (Hale et al., 2014), consequently the behaviours of the key species are often more important for biogeochemical processes than species diversity (Mermillod-Blondin and Rosenberg, 2006; O'Reilly et al., 2006). Consequently, the loss or reduction of the key bioturbators may negatively influence the intensity of bioturbation activities, thus lead to broad ecological and biochemical implications such as the declined nutrient fluxes attributing to less primary production (Lohrer et al., 2004b). Secondly, in the intertidal zones, the consistent positive relationships between bio-irrigation and SCOC were apparent in the dataset from baseline survey (**Chapter 2 and 3**) and density - dependent experiment schemes (**Chapter 4**; Figure 4.5a, 4.5b), implying that bio-irrigation is one of the main drivers of sediment respiration in the Scheldt estuary.

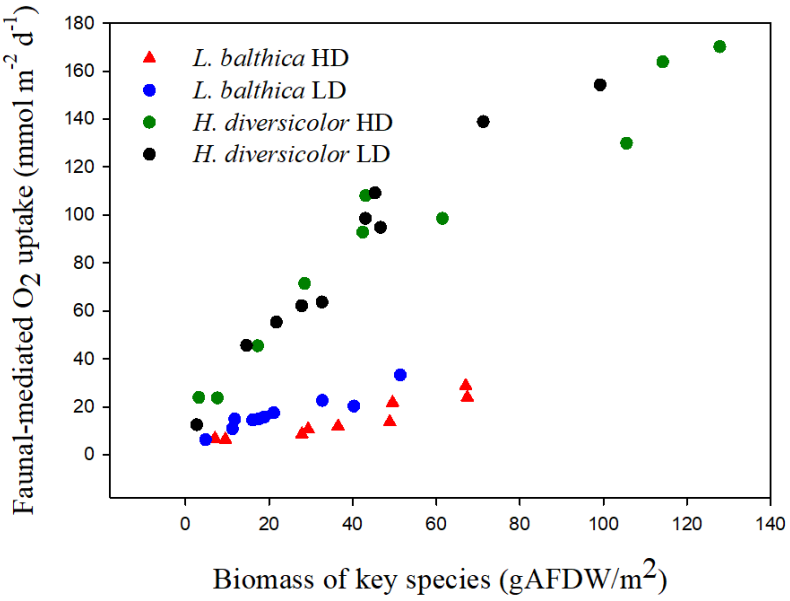


Figure 6.3: Relationship between faunal-mediated O_2 uptake and biomass measured in density-dependent experiment schemes with the key species (*L. balthica* and *H. diversicolor*) incubated in monoculture. HD – high dynamic habitats, LD- low dynamic habitats.

The results of the seasonal baseline survey confirmed that *H. diversicolor* and *L. balthica* play an important role in particle reworking and bio-irrigation in the Scheldt estuary (**Chapter 2**). The regression models confirm the significant contribution from *H. diversicolor* to another particle mixing attribute (i.e. maximum penetration depth of luminophores) at estuarine scale (methodology refer to **Chapter 2**; Table 6.1). Given the wide distribution of these two strong bioturbators on a great variety of sediments and their strong bioturbation activity (**Chapter 3**), both species are expected to substantially contribute to benthic biogeochemical cycling. The mechanisms underpinning this benthos-biogeochemistry relationship were further explored with manipulative laboratory experiments (**Chapter 4**). Despite the clear difference in sediment reworking and ventilation rates, biomass remained a fundamentally significant predictor across two target organisms with different biological traits by explaining 59% - 98% of the variance in oxygen dynamics in different habitats, particularly for *H. diversicolor* more than 95%

of the variance in faunal-mediated O_2 uptake can be solely linked to the variation of biomass (**Chapter 4**). The manipulative experimentation confirmed the biomass scaling of faunal-mediated O_2 consumption, and the biomass-dependent functions in predicting faunal-mediated oxygen consumption (**Chapter 4**) can be expected to be generalized, since metabolic rates of component species ultimately control many aspects of ecosystem functioning (O'Connor, 2009).

Table 6.1: Contribution of key species to the maximum penetration depth of luminophores, quantified with linear regression models (**Chapter 2-3**). R^2 values are given for each model. Data was derived from the baseline survey.

Month/predictor	Maximum penetration depth regression	Adjusted R^2	R^2	P
March				
x1: H. diversicolor	=1.04*HdiversicolorAFDW*+3.56	0.45	0.48	0.007
x2: Oligochaeta	OligochaetaAFDW+1.37			0.001
June				
x1: H. diversicolor	=0.73*HdiversicolorAFDW*+1.52	0.26	0.28	0.001
September				
x1: Oligochaeta	=4.57OligochaetaAFDW+2.07	0.28	0.30	0.001
December				
x1: H. diversicolor	=0.88*Hdiversicolor*AFDW+2.74	0.11	0.14	0.03
All				
x1: H. diversicolor	=0.51*HdiversicolorAFDW*+2.07	0.21	0.22	<0.001
x2: Oligochaeta	ligochaetaAFDW+1.96			0.001

Spatial-temporal variability exhibited in coastal and marine areas is mirrored in the benthos where distinct macrofaunal assemblages are associated with the changes in hydrodynamical, morphological and chemical features over a wide range of estuarine scales (Esselink and Zwarts, 1989; Ysebaert et al., 2003). However, it is not feasible to measure all the key controlling environmental variables and processes which are essential to regional assessments at all scales (Zhang et al., 2004). Field studies often result in limited datasets relative to the intrinsic spatial and temporal variability and the spatio-temporal scatter of observations, methodological differences between studies. Statistical models which identify the relationships between biological and environmental variables have much potential for applications in conservation biology and ecology (Guisan and Thuiller, 2005), because they can be developed when the underlying mechanisms are complex and difficult to unravel (Thrush et al., 2005). Species distribution models identify associations between environmental variables and the geographic distribution of a species, therefore can be linked to extrapolate the bioturbation effects at landscape scales based on robust population density-biogeochemistry relationships.

The development of assessment tools for ecosystem-based monitoring and management is becoming increasingly important and management focus shifts to address issues of loss of function and concomitant ecosystem services (Knights et al., 2014; Needham et al., 2011) with the growth of global anthropogenic pressures on natural environments (Halpern et al., 2008; Knights et al., 2015). So far, there has been no large-scale assessment of macrofauna-mediated ecosystem functioning in the Scheldt estuary, though continuous anthropogenic activities in the study site might induce large scale changes in the benthos-ecosystem functioning relationships. With the mechanisms of dominant benthic drivers in altering ecosystem functioning revealed across different

biotic and abiotic settings (i.e. context-dependent influence and population density effect) by integrating observational field fata (**Chapter 2-3**) and density manipulative experimental data (**Chapter 4**), novel parameterization of bioturbation effect from biomass of key species on sediment oxygen sediment dynamics was developed. It can contribute to the scalability of complex ecological relationships between coastal marine ecosystem functioning and macrobenthos. In light of the findings from **Chapter 2-4**, it is promising to upscale macrofauna-mediated ecosystem functioning by integrating spatial mapping of key bioturbators, thus providing greater insights into the range of responses to broad-scale anthropogenic stressors in naturally heterogeneous environmental settings. To upscale ecosystem functioning mediated by key bioturbators based on small-scale experimental studies, a case study adopting a 2-step model integration is proposed in **Chapter 5** (Figure 6.4), with *H. diversicolor* as the target organism and faunal-mediated oxygen uptake as the extrapolated ecosystem functioning process. This case study is to show how the use of species distribution modelling that builds on a robust understanding of ecosystem functioning allows the environmental (e.g. geomorphological) effects of anthropogenic and/or natural change of an estuarine system to be predicted with confidence. In the Western Scheldt, hydrodynamic changes induced by anthropogenic activities were found to negatively affect the spatial distribution and biomass of *H. diversicolor*, whilst numerous indirect effects are likely to reverberate throughout the ecosystem.

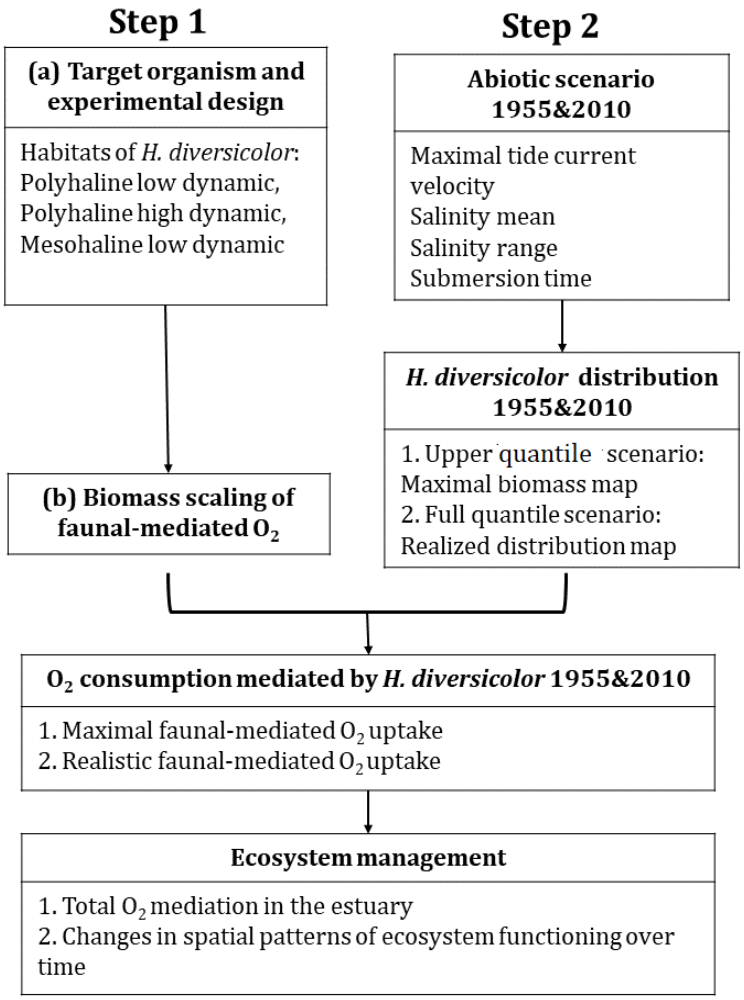


Figure 6.4: Schematic of the 2-step model integration (**Chapter 5**). Experimental measurements and biomass-scaling of faunal-mediated O_2 uptake (Step 1) is composed of (a) Target organism and experimental design and (b) biomass-dependent faunal-mediated O_2 uptake; Step 2 is to extrapolate ecosystem functioning over quantile regression model by adopting a mixed modelling approach with two temporal scenarios 1955/2010.

An interactive tool (R shiny app) with higher resolution of the model output was developed, for informing species management and anthropogenic alteration (e.g.

dredging-dumping practices) (for details please refer to **Chapter 5**; screenshot can be found below, Figure 6.5). The application can be downloaded via the following link:

https://drive.google.com/drive/folders/1hOFkhs813zfQ095hPR9RkrC2jywE1ZE9

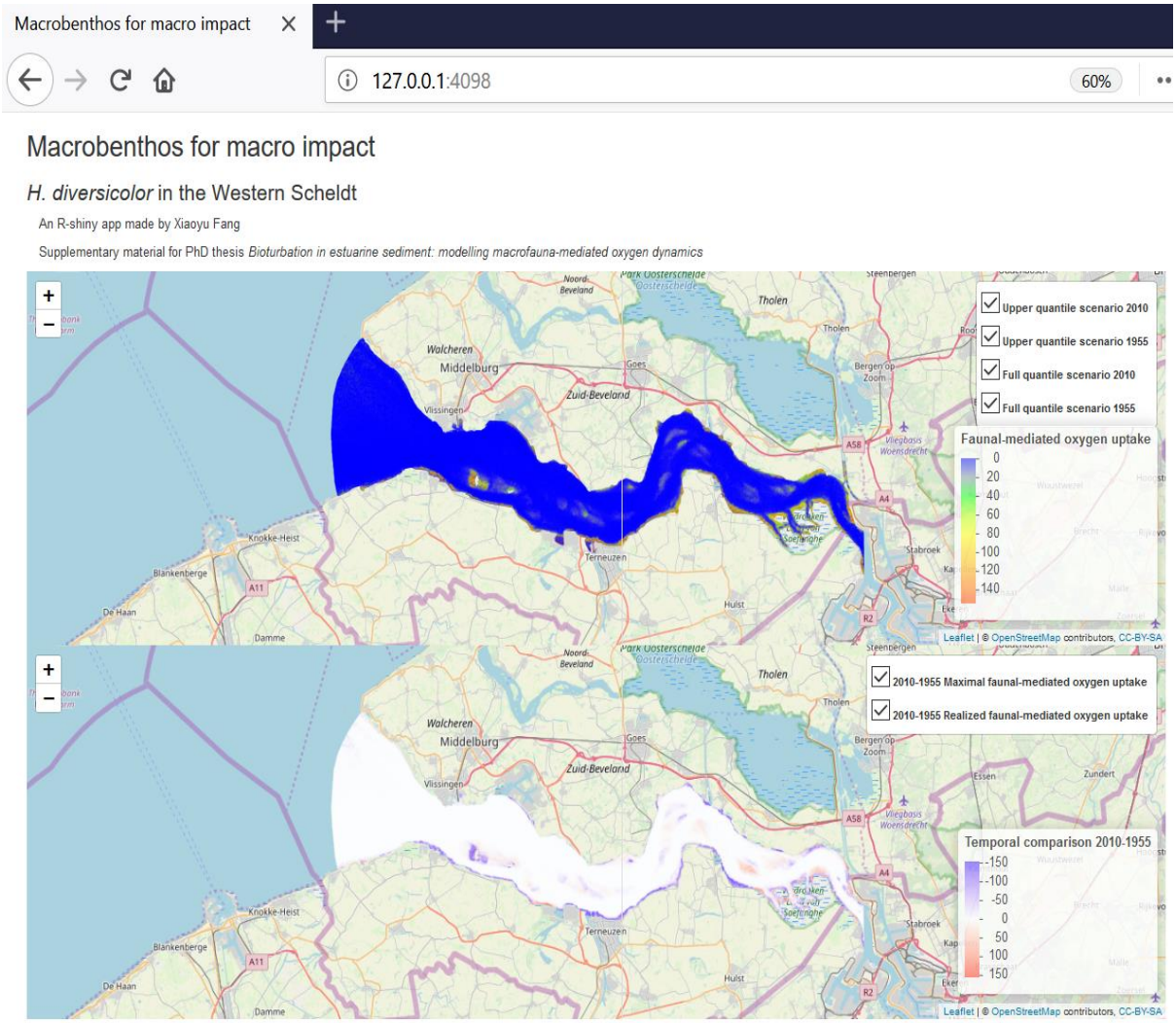


Figure 6.5: Screenshot of Rshiny app with interactive maps of faunal-mediated O_2 uptake at estuarine landscape level (**Chapter 5**)

6.3 Reflections and outlook

In this thesis, the implications of bioturbation effects on estuarine biogeochemistry were inferred from observational and experimental studies conducted in a range of environmental contexts, and small - scale ecosystem functioning experiments in exploring the dominant benthic drivers of functioning shift across scales. The present PhD study has highlighted the complex controls exerted by the naturally heterogeneous environmental settings and broad - scale anthropogenic stressors on the biogeochemical effects of bioturbation. As the first attempt towards bridging small-scale experiments with the broad-scale needs of ecosystem management, some limitations remained in uncovering the complexity of bioturbation impact on sediment biogeochemistry due to time and data constraints. These issues constitute important topics to the field of benthic biogeochemistry and estuarine ecosystem management, which are referred here as suggestions for future research directions.

6.3.1 Evaluation of experimental methodologies

The experimental results presented in this study were performed in a climate-controlled room and consequently it incorporates limitations inherent to laboratory-based studies. Such mesocosm and microcosm experiments hold the advantage that environmental conditions can be controlled so that the effect of the target organisms on ecosystem functioning can be evaluated without confusion caused by other variables. Additionally, these mesocosms and microcosms are easy to experiment upon, and more affordable and less labour-/time-intensive compared with *in situ* experiments, especially in assessing ecosystem functions and processes occur over a wide range of spatial and temporal scales. They can, however, fail to recreate the complex natural living environment of marine organisms. For instance, in this PhD study, the experimental set-up did not consider the potential effect(s) of interspecific interactions, tidal inundation time, day-night light and

temperature differences and hydrodynamic regime. Here, particle reworking and bio-irrigation were found to be low in the subtidal habitats (**Chapter 2**), although this result could be an artefact of lab conditions given the high current velocity *in situ.* Nonetheless, the experiment was set up with a focus on macrofauna-mediated measures (**Chapter 2-3**). In the Scheldt estuary, the macrobenthos occurred in low densities in the sampled subtidal habitats, therefore we mimicked the natural condition approximating to intertidal habitats allowing the mediation from macrobenthos to be quantified without potential masking of effects by strong hydrodynamics. With the difference between natural environment and the climate-controlled condition acknowledged, the parameterization obtained from laboratory-based experiments such as presented in this PhD thesis can be extrapolated to the natural environment approximating the laboratory-controlled environment. As in **Chapter 5** of this thesis, the faunal-mediated O_2 uptake was only extrapolated in intertidal areas in the Western Scheldt.

Even though the addition of artificial substrate such as luminophore to the environment might affect the underlying sediment community further influencing the fluxes measured, luminophores have been widely used in bioturbation research since they do not behave differently compared to the surrounding sediment (Mahaut and Graf, 1987). Besides, the use of frozen mud cakes has been approved in many laboratory-based and in situ experiments and no negative effects were reported in terms of activity and mortality being reported (e.g. De Backer et al., 2011; Braeckman et al., 2010; Montserrat et al., 2009). Visual observations in all experimental units in this PhD study suggest the resident fauna mixed the mud cake with the underlying sediments, which would reduce the chemical differences between the natural and the processes sediment. Additionally, all the organic matter was previously removed by muffling in pre-treated sediments and the mud cakes were frozen for a limited time. Due to the practical difficulties in maintaining

the mud cake with the same size as the entire surface of the large cores (**Chapter 2-3**), the covered surface 13x13 cm has been the maximum surface area which can be adopted to ensure the bioturbation measurement and the successful application of mud cakes. During the experiment, the potential bias has been recognized so the small cores used to subsample for slicing were all positioned at the centre of the big cores.

While mostly contributed by macrofauna, bioturbation is also influenced by meiofauna and microbenthic assemblages. Densities and diversity of microbiota and meiofauna were not assessed in this study, however, the low goodness of fit of the model and low proportion of constrained variance (**Chapter 3**) and the low positive bio-irrigation rates measured in the sediment cores collected from subtidal habitats without the presence of macrofauna (**Chapter 2**) suggest that other biotic factors are also at play. Meiofauna can strongly influence biogeochemical fluxes and sediment processing through bioturbation or grazing on micro-organisms or sediment stabilising substances (Schratzberger and Ingels, 2018). Additionally, meiofauna can facilitate mineralisation of organic matter, concomitant nutrient regeneration and affect bacterial communities (Bonaglia et al., 2014). They can also interact with macrofauna through predation, competition, or modifying the habitats (Coull, 1999). In the presence of bioturbating and bio-irrigating macrofauna, a net enhanced meiofaunal diversity has been observed (Ólafsson, 2003). Therefore, the exclusion of the bioturbation effect of meiofauna might lead to inaccurate estimations given their important role in regulating benthic fluxes. In the Scheldt estuary, the distribution of meiofauna was found to be positively related to grain size, sediment pigment concentrations and salinity (Gallucci et al., 2005; Moens et al., 1999). So high diversity of meiofauna may occur in polyhaline zone high-dynamic habitat, which cause the low fit of multivariate model predicting benthic fluxes based on environmental and macrofaunal descriptors in high-dynamic habitat (**Chapter 3**). Another factor that could

contribute biogeochemical cycling is change in the density and diversity of micro-organisms; differences not considered here. The distribution of microbes is attributable to abiotic factors such as temperature, light, salinity or nutrient concentrations (Bernhard et al., 2005). Therefore, their relative contributions to the variability in biogeochemistry may change according to the habitat or season. In the baseline survey (**Chapter 2-3**), and improved performance of the models that incorporated season- or habitat-defined subsets of the total dataset a need to consider spatio-temporal variability. The distribution of macrofauna is associated with microbenthic assemblages. For example, macrofauna creates new microniches inside the sediment with different physical structure and chemical composition from the surroundings by reworking sediment particles and ventilating burrows (Bertics et al., 2010). Due to the extension of the liveable habitats, the surface-dwelling micro-organisms would live deeper in the bioturbated sediments (Laverock et al., 2011). As a result, higher microbial density or diversity would occur and the extended reach of their metabolism and the associated biogeochemical fluxes would be expected. In the Scheldt estuary, the positive correlation between the coarse sediments and microbial densities and diversity has been observed, which could be explained by the higher internal sediment surface in sandy sediment (Herman et al., 2001). As for the seasonal survey in this PhD research (**Chapter 2-3**), the diversity and densities of microbiota might peak in the polyhaline high-dynamic intertidal habitats in June or December when particle reworking and bio-irrigation rates were high. Given the correlation between the distribution of meio-, micro- and macrofauna and similar environmental determinants in predicting meiofauna and microbiota, any bias in the models built in **Chapter 2-4** is likely to be modest. However, it is less clear how the associated diversity and densities of meiofauna and microbiota translate to the biogeochemistry of estuarine sediments. For future studies with a focus on the relationships between bioturbation, bio-irrigation or benthic fluxes, the densities

and diversity of meiofauna and microbiota should be taken into consideration, especially in the habitats where high bioturbation rates were observed in this PhD study.

Additionally, the replication (n=4) was low in the baseline survey (**Chapter 2-3**), which might account for absence of difference. The replication ("n") of each treatment was limited due to logistic constraints inherent to the use of a laboratory system. The absence of statistical differences in biological responses sometimes observed in this work (**Chapters 2-4**) may derive from low statistical power, as well as the variability in individual responses within a treatment. Nevertheless, studies focusing on effect size of biological responses provide useful information and should not be disregarded (Cornwall and Hurd, 2015).

6.3.2 Improvements in laboratory-based experiments and model integration

6.3.2.1 Baseline understanding of benthos-environment relationship - Chapter 2,3

The baseline understanding of benthos - environment relationship in the Scheldt estuary can be improved by integrating fine-scale experimental studies (e.g. site-specific, species-specific). Although the baseline survey has been designed to encompass a wide range of environmental contexts (i.e. main seasonal differences, salinity zones and habitats), the contributions of environmental and macrofaunal properties to the benthic biogeochemistry varied in each habitat and season. In **Chapter 3**, the relatively low fit of the models predicting biogeochemical fluxes in some habitats (subtidal and high-dynamic intertidal habitats) and months (September and December) imply that the results obtained from studies have not sufficiently incorporated estuarine heterogeneity, which might lead to inaccuracy in interpreting spatio - temporal patterns of ecosystem functioning. Therefore, heterogeneity should be accounted for when the results from the baseline survey are interpolated to the entire estuary. A refined sampling strategy across

multiple sites and years can be developed based on the current sampling framework, to maximize the spatio-temporal heterogeneity whilst minimize labour-intensive execution. In this study, habitats were chosen based on the classification of hydrodynamics, although other abiotic characteristics differ (e.g. grain size and mud content). More habitat types can be included such as the tidal marshes. These are also important habitats associated with nutrient cycling and trapping of organic matter, which probably exhibit different environment, biodiversity and ecosystem function relationships. The temporal resolution can also be improved, given the sampled months in this PhD study was limited over the course of one year, neglecting some ecological events, such as the spring bloom. It is highly recommended to put focus on improving the observational extent and consistency when decide sampling sites and frequencies. In studying species-ecosystem interactions, referring to ecotope map is of great help in deciding sampling sites; the measurements of biotic community identification and ecosystem processes and functions can be combined with abiotic measurements to maximize the chance for gaining mechanistic insights. The integration of both local- and macroscale data can provide a robust mechanistic understanding of bioturbator-ecosystem functioning relationship, and further ensure a firm predictive framework in a more realistic context.

6.3.2.2 Microcosm-derived model parameterization of key bioturbators' effect on sediment oxygen dynamics - Chapter 4

The quantitative understanding of key bioturbators' effect on sediment oxygen dynamics was derived from a simplified system (**Chapter 4**). Despite that the density treatments and experimental set-up were designed within naturally experienced conditions, the experiments were entirely conducted in a laboratory, and consequently it incorporates limitations inherent to laboratory-based studies. For the set-up of microcosms, measuring fluxes in permeable sediments isolated from advective transport mechanisms

underestimates the mineralisation of organic matter, because it does not include the influence of the removal of decomposition products generated by water currents (Ehrenhauss and Huettel, 2004). The key bioturbators were incubated in monoculture, therefore the biotic interactions in the benthic assemblages were not considered. The experimental studies provide essential data for understanding the species' mechanisms of *L. balthica* and *H. diversicolor* (**Chapter 4**), nevertheless, the biomass-dependent parameterization of bioturbation effect on SCOC built in this study could be a consequence of a relatively short maintenance period, and perhaps is limited to an extent by the amount of data available. To develop a comprehensive picture of how macrofauna species interact in the sediment environment, density-dependent experiments like this study but with a focus on other key bioturbators in different sediment types would lead to detailed assessment in species - specific effects thus a more precise modelling of benthic oxygen fluxes. Experiments looking at changes in different species combinations matching those carried out in **Chapter 4** could also be undertaken. As the underpinned mechanisms of faunal-mediated oxygen dynamic cannot be directly translated to other biogeochemical processes, such as nitrification-denitrification, similar manipulative experimentation on other ecosystem functioning processes (e.g. nitrification - denitrification) are highly recommended.

6.3.3 Evaluation of modelling framework

Given the importance of knowledge of species distributions for conservation and climate change management, major progress has been made in improving the prediction of species' range limits and habitat suitability (reviewed in Kearney and Porter 2009). There are several approaches available. Correlative approaches (e.g. logistic regression) statistically link species distribution records to environmental data and require little knowledge of mechanistic links between biota and the environment (Guisan and

Zimmerman, 2000). Alternatively, mechanistic approaches can be used to link biological functional traits and the environment, for instance, to map population dynamics of organisms (Dormann, 2007).

Here, we used correlative quantile regression to compare differences in the spatial distribution of macrobenthos performance (i.e. mediated oxygen uptake). Species distribution are constrained by different environmental variables, even when the known factors are not limiting, other unknown factors might be limiting the presence or abundance of the organisms (Thrush et al., 2005). As a consequence, the spatial distribution of organisms is heterogeneously scattered below an upper boundary instead of around a central value. Central estimators fail to simulate data sets where the variance changes with the mean (Cozzoli et al., 2013). Quantile regression copes with the heterogeneity caused by such unmeasured factors by modelling not the mean but the upper (or lower) quantiles (Cade et al., 2003). A major strength of quantile regression is its consistency with ecological theory (Thrush et al., 2005). Changing the quantile allows assessment of spatial fluctuation in the relationships between environment and biota (Table 5.1; Supplementary material of **Chapter 5**). For instance, the upper quantile scenario expresses species distributions in terms of potential niche or habitat suitability (Franklin, 2010) and is valuable for predicting a static boundary for habitat suitability despite potentially high environmental variability (Degraer et al., 2008). In contrast, a full-quantile model provides wider context and comparison of different ecosystems by generating predictions of broadscale environment-biota relationships.

The focus of this study is the response of estuarine ecosystem to the past and current anthropogenic influence. In the Western Scheldt, variations in hydrodynamics due to human landscape alterations (e.g. dredging) are known to be the main source of habitat changes in recent decades (Smolders et al., 2013). The changes in maximal tidal current

velocity, inundation time and salinity were chosen as predictor variables, because they are known to be among the most important hydrodynamic variables in determining coastal and estuarine benthos distribution (Ysebaert et al., 2002). Besides, large scale scenarios are available from hydrodynamics models, thus they can be used to extrapolate species distribution models prediction on a whole basin scale. Hydrodynamic models can provide scenarios for different periods of the ecosystem evolution, allowing historical time comparisons (like in the present study) and forecasts about future ecosystem development (Smolders et al., 2013).

In this study, we used a modelling approach to assess the bioturbation effects of macrobenthos on oxygen dynamics across an estuarine landscape. Empirical studies have highlighted the important role of biota across regional landscape scales (Norkko et al. 2015), but also that the spatial arrangement relative abundance (dominance) of species can greatly influence the contribution of bioturbators to ecosystem functioning (Wohlgemuth et al., 2017). It is well known that changes in species' abundance, distribution and behaviour in response to global environmental changes can lead to significant alteration of ecosystem function and services (Godbold et al., 2013) and Snelgrove et al. (2018) argues for the need to incorporate biological processes into to global geochemical models to improve projections of the impact of global environmental change on functioning and deliver better societal decisions on ecosystem management.

6.3.4 Application potential and logical next steps

The faunal-mediated O_2 uptake mapping presented in **Chapter 5** indicate that integration of species distribution models and biomass-dependent models can offer a simple way to assess ecosystem functioning from a benthic perspective. The mixed approach of experiments and model integration scales the bioturbation effect from single individuals

to population and landscape levels. It is of ecological and social applicability and can be generalized to other management cases in other estuarine ecosystems because (1) the abiotic and biotic predictors are general descriptors which can be easily acquired and (2) different abiotic-biotic scenarios can be developed by tuning the desired quantiles. However, the local customization is necessary since the direct consequences of human activities vary in different estuarine ecosystems. Therefore, the local environmental condition should be considered in the selection of abiotic predictors to highlight the anthropogenic influence on species distribution. Insights into the possible alterations to the provision of ecosystem services can support decision-making towards sustainable management of the marine environment (Henson et al., 2017; Knights et al., 2014), however it is complicated to establish management plans of the estuarine ecosystem because of the highly dynamic characteristics (i.e. continuously changing currents, ongoing sedimentation/erosion processes) and the complex feedback loops between abiotic and biotic factors (Bouma et al., 2007). Estimation of ecosystem functioning is challenging due to (1) context-dependent benthic mediation leading to spatial variation of sediment metabolism generated by same species, and further complicated by (2) different environmental constraints acting at different scales in determining distribution of organisms (Morais et al., 2016). Thus, quantifying the contribution of benthic habitats to regional and global carbon and nutrient cycles is difficult because of the uncertainties in scaling biogeochemical measurements made in small patches (cm^2) to whole ecosystems (km^2) (Delmotte et al., 2008). Scale can be important for different variables (Archambault and Bourget, 1996; Godbold and Solan, 2013), and there may be critical scale thresholds for estimating biogeochemical dynamics and scale-dependent cascading effect of influence between variables (e.g. Guichard and Bourget 1998). Besides, the integration of species distribution models and biogeochemical models would increase the uncertainties.

In the Scheldt estuary, *H. diversicolor* is a good model organism not only because it is a key bioturbator (**Chapter 2**) and constitutes a large portion of total biomass of macrobenthos community (**Chapter 3**), but also due to the shared parameterization of its bioturbation effect across different habitats in the studied estuary (**Chapter 4**). The experiment-derived parameterization of *H. diversicolor* implies the potential to extrapolate the faunal mediation on oxygen dynamics to an estuarine landscape level. Additionally, individuals of *H. diversicolor* are easy to be harvested by hand and maintained in laboratory, so they serve as a good biological material for experimental studies (Scaps, 2002). With the microcosm-derived results, our qualitative and mechanistic understanding of the bioturbation effect from single species can be increased. Nonetheless, it is essential to account for species interaction when extrapolating mesocosm experiments to field contexts (Kristensen et al., 2013), considering the population-and context- dependent contributions from species to ecosystem processes and functions (**Chapter 2-3**). Thus, the next logical steps are to include the dispersion and biotic interaction (e.g. are competition, predation, parasitism and mutualism). The dispersion of organisms can be integrated based on the dispersion abilities of the modelled species and seasonal variation in environmental variables (e.g. water temperature maps in different seasons) (Adiaensen et al., 2003). The various biotic interactions that can be included are competition, predation, parasitism and mutualism (Vaughan and Ormerod, 2003). Such efforts have been made by Sundermeyer et al. (2005), the distribution of fish was predicted by explicitly incorporating dispersion limitation and biotic interactions. For example, the predators or prey of the modelled species can be simply included based on trophic interactions. However, since the species densities are often direct and causal predictors (the density of one species is the predictor for another), the use for extrapolated would be limited. Another challenge posed for the integrated model is that many biotic interactions are local and idiosyncratic (Kearney and

Porter, 2009). Therefore, the effects of the species on faunal-mediated O_2 uptake in a mixed macrobenthos community are more complex, depending on how interspecific interactions affect the activities of the involved species.

Broad general rules allow for application in a wide range of environmental conditions. Another step forward would be to see if the identified relationships between environment, macrobenthos and ecosystem function hold in another system or context. The non-static nature of species-environment relations has been recognized (Belley et al., 2017), implying different local environmental conditions can affect distribution of bioturbators and the outcome of benthos-mediated oxygen uptake. Thus, data from different ecosystems should be assessed regarding the biotic contributions to sediment oxygen dynamics. So that various abiotic-biotic relationships derived from different systems can be included in the current model (**Chapter 5**) to increase the predictive performance. Meanwhile, other abiotic variables which have not been considered in this PhD study, such as sediment granulometry, temperature, primary production and interaction with invasive species, should be incorporated into the species distribution models.

Seawater temperature is widely recognised as a key regulator of metabolic rates in macrofaunal invertebrates (Gillooly et al., 2006; Brown et al., 2004), thus incorporating temperature into benthos-mediated oxygen dynamics models can help to explain seasonal, latitudinal and global climate changes in biotic contribution to sediment community oxygen consumption. The biomass-dependent model parameterization was derived under the experimental temperature 15 °C (**Chapter 4**). This was decided based on the consideration that the biotic contribution was higher in warmer months (March to September) in the baseline survey (**Chapter 2-3**) and the average water temperature in the Scheldt estuary from March to September from 1995 to 2001 is 15 °C (Van Damme

et al., 2005). A range of temperatures were not considered here due to logistical constraints, nevertheless, general ecological theory grounded in metabolic scaling (Brown et al., 2004) can be used to extrapolate these results to allow fluctuations in temperature and biomass (see **Chapters 4-5**) to be incorporated within the modelling framework. The positive relationship between bioturbation activities and temperature has been confirmed by previous studies (Kristensen et al., 1983; Baranov et al., 2016). To illustrate, I performed a simple extrapolation using metabolic scaling. Metabolic scaling coefficients were calculated according to the general theory of Gillooly et al. (2001), described using the following equation: $R \sim M^{a} \times e^{-E/kt}$, where R is faunal-mediated O_2 uptake (i.e. bioturbation effect), a is the scaling exponent for mass, M is body mass, E is the activation energy, k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}), and T is the body temperature in Kelvins (or water temperature for aquatic invertebrates). A general interspecific mean activation energy of 0.65 eV was previously calculated for both marine and freshwater species (Gillooly et al., 2001; Brown et al., 2004). In the Scheldt estuary, the experimental temperature was 15 °C. In the baseline survey (**Chapter 2-3**), the water temperature of Western Scheldt in warmest (September) month was 20 °C, and the lowest water temperature was 6 °C in March. According to the metabolic scaling, the high temperature of 20 °C and low temperature of 6 °C would generate an increase of 56% and a decrease of 57% in the metabolic rates for same population of bioturbators, when all other mechanisms involved in oxygen fluxes do not change with temperature variability. Besides, other factors influence metabolic rates of macrobenthos should be taken into consideration such as depth, sex and age (Clark and Behmer, 2016).

Chapter 7 General conclusions

In **Chapter 2**, the key species' mechanisms were revealed by exploring spatio-temporal variation in sediment ecosystem processes (e.g. particle mixing and bio-irrigation) and identifying key benthic contributors. While macrobenthos with different functional traits presented different particle mixing modes, biodiffusion dominated sediment reworking as a transport mechanism. Both particle reworking and bio-irrigation were highly variable among seasons and habitats, and different species were found to contribute unevenly to both processes, and between habitats and seasons. Habitat structuring effects on populations, density-dependent interactions with the habitat, and temperature-driven variability in macrobenthos activity and living position are suggested to explain the observed spatial and temporal differences.

Chapter 3 is focused on the macrobenthos-biogeochemistry relationship, and the spatio-temporal patterns of biogeochemical fluxes in the Scheldt estuary were illustrated to be the compound effect of the biotic regulation by population density of macrobenthic community and the activities of macrobenthic functional groups (e.g. particle mixing and bio-irrigation), and the abiotic gradients along the estuary and seasonal variability. The macrofaunal functional roles in biogeochemistry varied throughout the different habitats and seasons, characterized by more contribution in low-dynamic intertidal habitats and less contributions in the subtidal habitats and high-dynamic intertidal habitats whilst environmental factors mainly determined the biogeochemical fluxes. The dominant functional groups were context-dependent, since the importance of certain groups shifted alternately.

The importance of habitat- and biomass-dependent influences on the benthos-ecosystem functioning relationship was further explored by experimenting on key bioturbators *H.*

diversicolor and *L. balthica* at different population density levels across the natural habitats in **Chapter 4**. The quantitative understanding of key bioturbators' effect was acquired, that faunal-mediated influences on sediment oxygen dynamics can be sufficiently predicted by biomass; whilst less variance was attributed to the habitat and species-specific influences.

Given the findings gained from the baseline survey (**Chapter 2-3**), and the biomass-scaling of faunal-mediated O_2 uptake of key bioturbator *H. diversicolor* (**Chapter 4**), an assessment tool for ecosystem-based O_2 dynamics was developed in **Chapter 5**. The contribution of *H. diversicolor* across a range of biomasses in sediment metabolism was quantified and the spatial variability of the faunal-mediated O_2 consumption was extrapolated in the Western Scheldt. Hydrodynamic changes induced by anthropogenic activities (i.e. dredging) are most likely to negatively affect the spatial distribution and biomass of *H. diversicolor*, whilst numerous indirect effects are likely to reverberate throughout the ecosystem.

To summarize, the understanding of how natural variability and anthropogenic disturbance affect benthic biogeochemistry is particularly important for shallow estuaries where whole ecosystem function can be dominated by benthic processes. For ecological conservation of key communities, habitats and ecosystem functions, a mechanistic understanding of spatio-temporal patterns of ecosystem functioning and how this matters for a range of ecosystem properties over long-term is necessary. The experiments carried out at small-scale can be promising to set up a robust mechanistic understanding for the subsequent extrapolation to the predictions landscape level. Results from this thesis suggest that local variability in densities of key bioturbators could cause ecologically relevant changes in sediment metabolism. The spatial distribution of organisms would therefore need to be accounted for in predictive models.

This thesis demonstrates the possibility to derive and model the bioturbation/ecosystem functioning patterns, where direct assessments are labour intensive and costly to be carried out routinely at landscape level. The multi-disciplinary approach applied in this thesis is promising to be widely adopted due to its ability to build mechanistic understanding of bioturbation effect on ecosystem functioning towards spatial mapping of benthic ecosystem functioning at different time scales.

Supplementary material

Supplementary material for chapter 2

Figure 2.1: The hierarchical spatial sampling design (baseline survey)

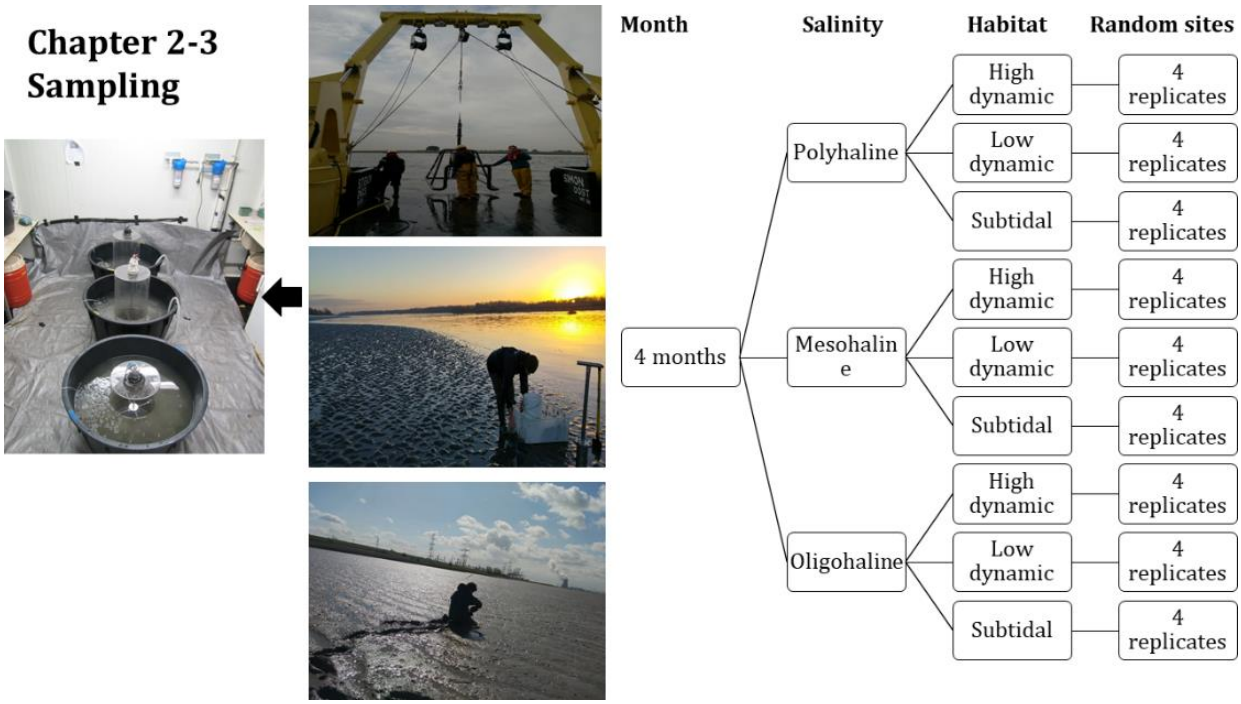


Figure 2.2: Photo of experimental set-up in climate-controlled room at Ghent University (Belgium).

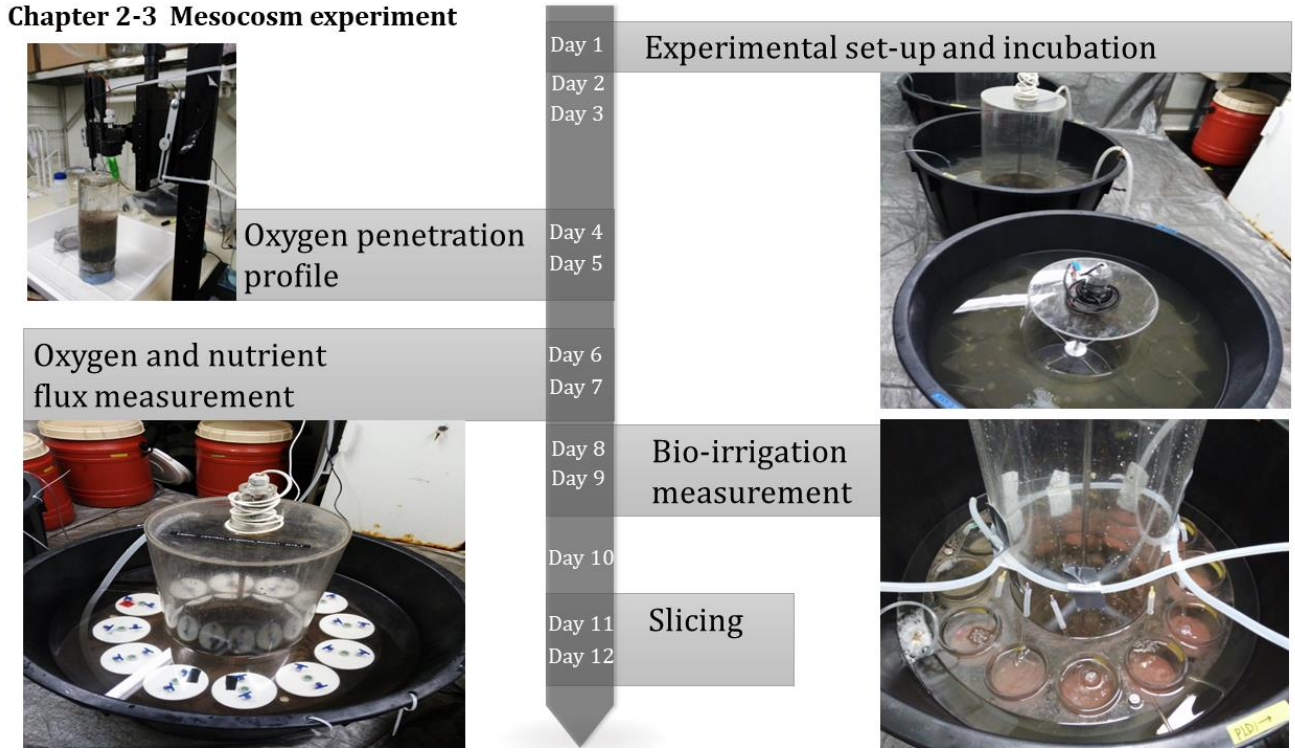
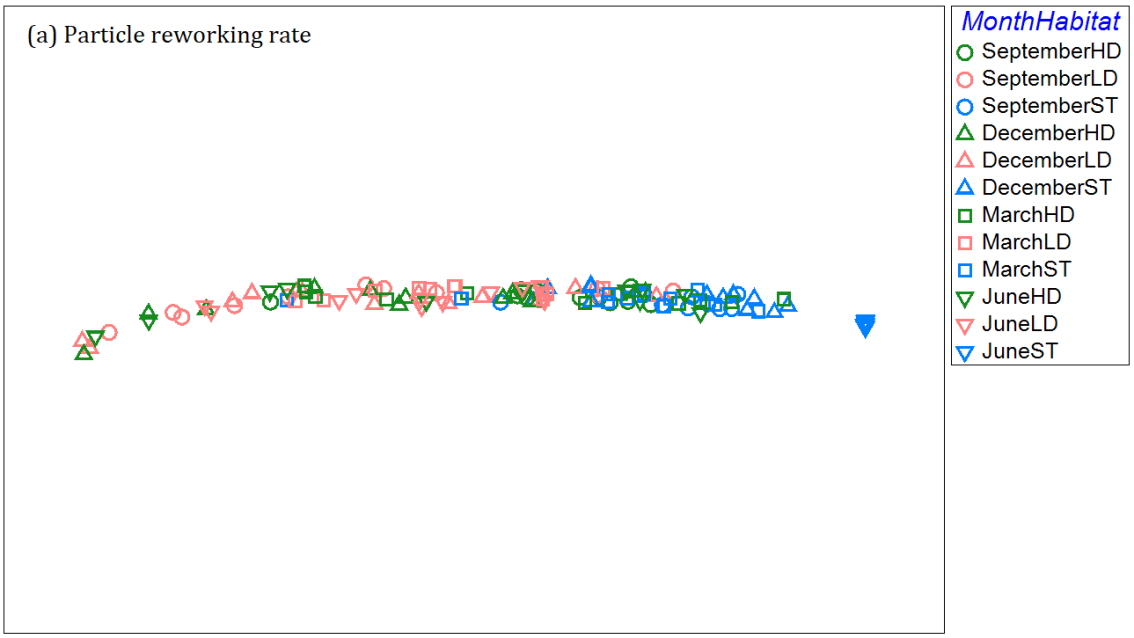


Figure 2.3: Non-parametric multivariate analysis MDS plots of (a) Particle reworking rate and (b) Maximum penetration depth.


(a) Particle reworking rate
MonthHabitat
SeptemberHD
SeptemberLD
SeptemberST
DecemberHD
DecemberLD
DecemberST
MarchHD
MarchLD
MarchST
JuneHD
JuneLD
JuneST

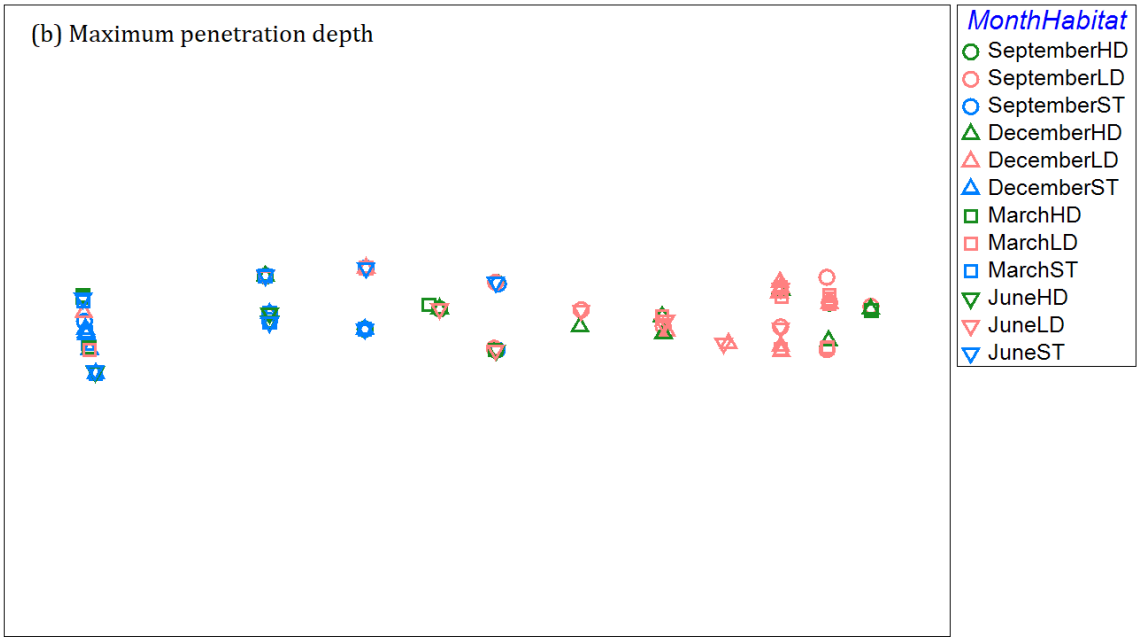
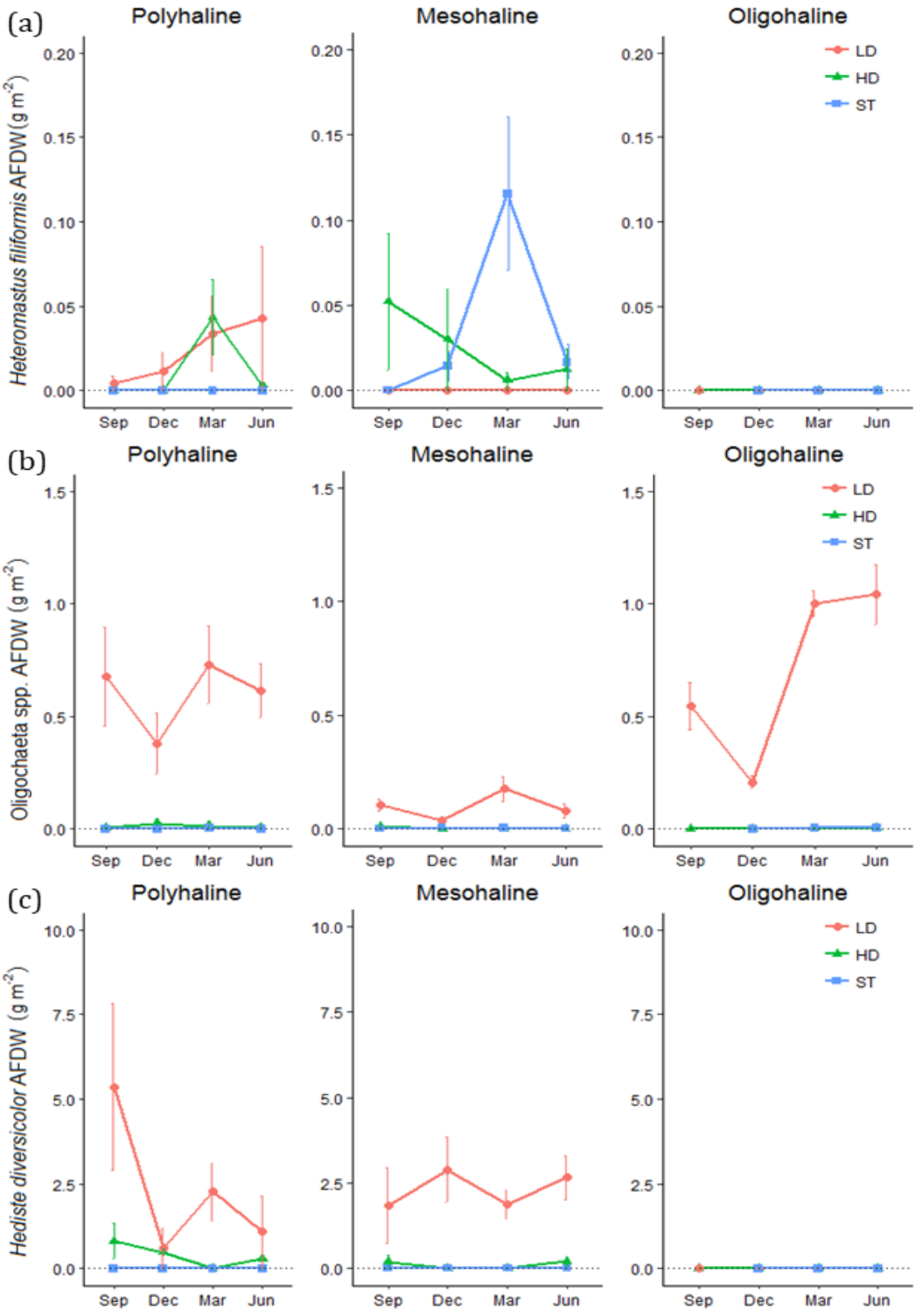
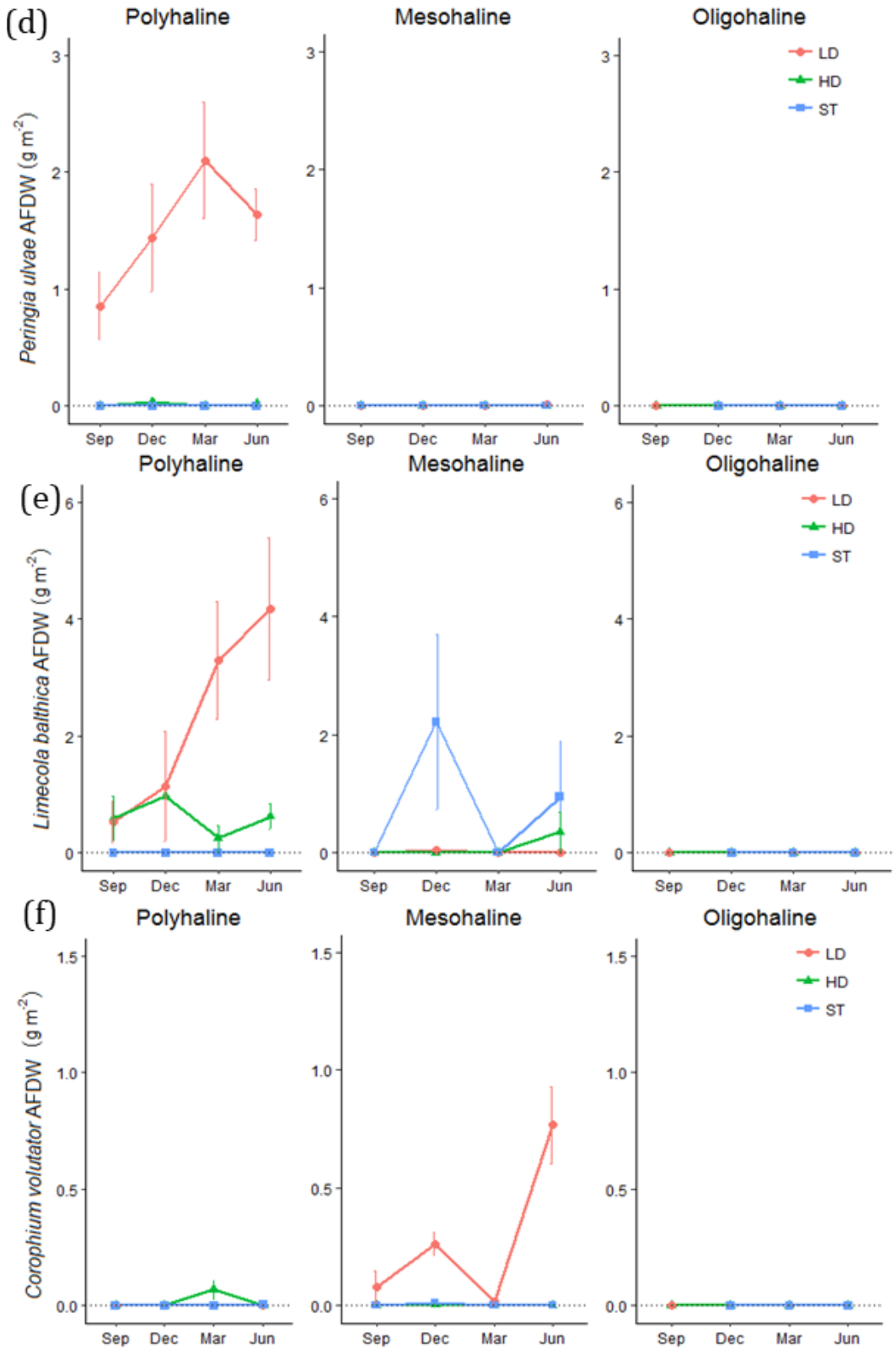

(b) Maximum penetration depth
MonthHabitat
SeptemberHD
SeptemberLD
SeptemberST
DecemberHD
DecemberLD
DecemberST
MarchHD
MarchLD
MarchST
JuneHD
JuneLD
JuneST

Figure 2.4: Spatio-temporal variability in biomass (AFDW: Ash free dry weight) of the key bioturbators: (a) *Heteromastus filiformis*, (b) Oligochaeta spp., (c) *Hediste diversicolor*, (d) *Peringia ulvae*, (e) *Limecola balthica*, (f) *Corophium volutator*, (g) *Corophium arenarium*, (h) *Eteone longa*, (i) *Bathyporeia pilosa* (ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal, Sep = September, Dec = December, Mar = March, Jun = June).


(a)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Heteromastus filiformis AFDW (g m-2)
0.20
0.15
0.10
0.05
0.00
Sep
Dec
Mar
Jun
(b)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Oligochaeta spp. AFDW (g m-2)
1.5
1.0
0.5
0.0
Sep
Dec
Mar
Jun
(c)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Hediste diversicolor AFDW (g m-2)
10.0
7.5
5.0
2.5
0.0
Sep
Dec
Mar
Jun


(d)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Peringia ulvae AFDW (g m-2)
Sep
Dec
Mar
Jun
(e)
Limecola balthica AFDW (g m-2)
(f)
Corophium volutator AFDW (g m-2)

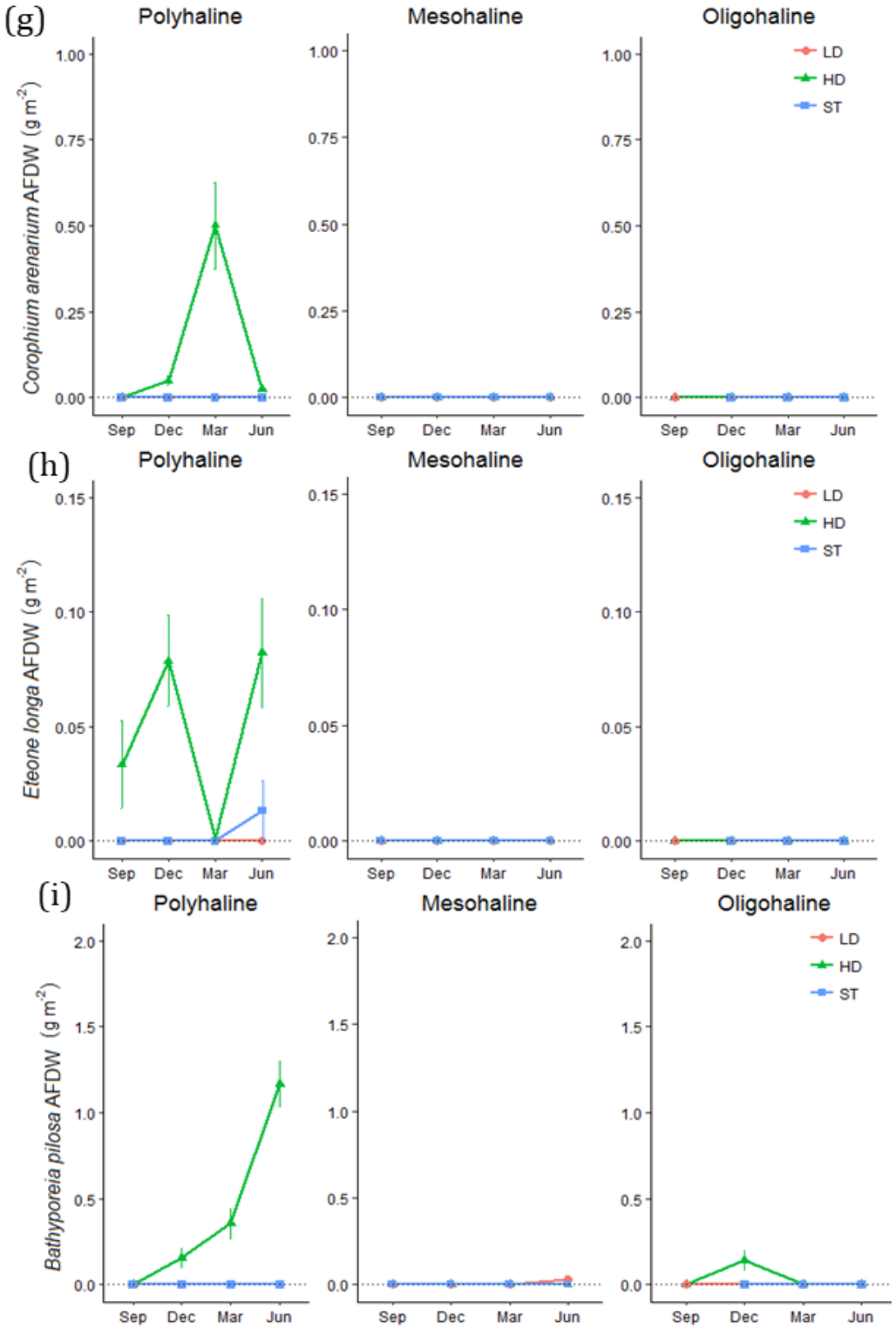

(g)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Corophium arenarium AFDW (g m-2)
1.00
0.75
0.50
0.25
0.00
Sep
Dec
Mar
Jun
(h)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Eteone longa AFDW (g m-2)
0.15
0.10
0.05
0.00
Sep
Dec
Mar
Jun
(i)
Polyhaline
Mesohaline
Oligohaline
LD
HD
ST
Bathyporeia pilosa AFDW (g m-2)
2.0
1.5
1.0
0.5
0.0
Sep
Dec
Mar
Jun

Table 2.1: Full linear regression models, analysing bio-irrigation as a function of species biomasses. y always represents bio-irrigation rates. HD = high-dynamic intertidal, LD = low-dynamic intertidal.

Month/predictor	Bio-irrigation regression equation	Adjusted R^2	R^2	P
March				
x1: Hediste diversicolor	y=1.67x1+49.55x2+2.59	0.51	0.54	<0.001
x2: Corophium volutator				<0.001
June				
x1: Hediste diversicolor	=0.68x1+3.82	0.16	0.19	0.014
September				
x1: Hediste diversicolor	=0.74x1+18.21x2+3.56	0.61	0.64	<0.001
x2: Corophium volutator				0.007
December*				
x1: Hediste diversicolor	=0.11x1+7.67x2+1.28	0.66	0.67	<0.001
x2: Corophium arenarium				<0.001
Habitat/predictor				
High dynamic				
x1: Hediste diversicolor	=2.6x1+105.81x2+2.28x3+2.65	0.53	0.57	0.003
x2: Oligochaeta				0.006

x3: Limecola balthica				0.003
Low dynamic		0.48	0.49	
x1: Hediste diversicolor	=0.7x1+4.53			<0.001
All*				
x1: Hediste diversicolor	=0.07x1+0.49x2+1.36	0.29	0.3	<0.001
x2: Corophium arenarium				0.003

* The bio-irrigation data for the total model and the model in December were fourth-root transformed before analysis, to meet the assumptions for linear regression

Supplementary material for chapter 3

Table 0.1: Overview of the mean values for all environmental variables ± standard errors. P = polyhaline, M = mesohaline, O = oligohaline. ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal. Temperature (T) is expressed in °C, Salinity (S) in PSU, Chlorophyll a (Chl a) in µg g^{-1}, median grain size (MGS) in µm, mud content in %, total nitrogen (TN) in % and total organic carbon (TOC) in %.

			T	S	Chl *a*	MGS	Mud content	TN	TOC
September	P	ST	20	30	0.68 ± 0.23	332 ± 13	6 ± 1	0.85 ± 0.15×10^{-1}	0.23 ± 0.04
		HD	20	30	9.40 ± 1.53	221 ± 0.06	3 ± 0.4	1.02 ± 0.17×10^{-1}	0.21 ± 0.05
		LD	20	30	4.56 ± 0.38	47 ± 0.5	64 ± 0.6	1.75 ± 0.10×10^{-1}	1.22 ± 0.05
	M	ST	20	15	0.62 ± 0.16	152 ± 5	21 ± 2	1.08 ± 0.01×10^{-1}	0.71 ± 0.08
		HD	20	15	1.16 ± 0.11	125 ± 2	21 ± 1	1.25 ± 0.20×10^{-1}	0.50 ± 0.02
		LD	20	15	4.00 ± 0.72	38 ± 0.6	71 ± 0.7	2.63 ± 0.12×10^{-1}	2.39 ± 0.05
	O	HD	20	2	0.49 ± 0.12	121 ± 2	8 ± 2	1.15 ± 0.02×10^{-1}	0.38 ± 0.06
		LD	20	2	5.66 ± 0.64	47 ± 1	64 ± 2	2.61 ± 0.08×10^{-1}	2.21 ± 0.02
December	P	ST	10	24	0.75 ± 0.14	196 ± 50	33 ± 7	0.63 ± 0.08×10^{-1}	0.62 ± 0.09
		HD	10	24	4.89 ± 1.09	227 ± 2	3 ± 0.5	0.16 ± 0.02×10^{-1}	0.21 ± 0.02
		LD	10	24	5.95 ± 0.88	46 ± 0.7	65 ± 0.4	2.81 ± 1.78×10^{-1}	1.24 ± 0.07

	M	ST	10	15	0.60 ± 0.16	174 ± 9	17 ± 4	0.76 ± 0.06×10^{-1}	0.56 ± 0.14
		HD	10	15	2.28 ± 0.48	108 ± 7	28 ± 4	0.77 ± 0.09×10^{-1}	0.53 ± 0.06
		LD	10	15	3.13 ± 0.36	30 ± 1	74 ± 1	2.70 ± 0.03×10^{-1}	2.79 ± 0.05
	O	ST	10	3	0.19 ± 0.03	253 ± 49	3 ± 0.9	0.53 ± 0.05×10^{-1}	0.21 ± 0.04
		HD	10	3	0.18 ± 0.02	147 ± 2	5 ± 0.5	0.58 ± 0.09×10^{-1}	0.17 ± 0.06
		LD	10	3	6.09 ± 0.71	47 ± 1	64 ± 0.9	1.90 ± 0.12×10^{-1}	1.70 ± 0.11
March	P	ST	6	20	0.78 ± 0.30	261 ± 21	18 ± 5	0.47 ± 0.08×10^{-1}	0.34 ± 0.11
		HD	6	20	6.37 ± 0.37	227 ± 2	5 ± 0.6	0.30 ± 0.02×10^{-1}	0.20 ± 0.06
		LD	6	20	7.20 ± 0.50	47 ± 0.3	63 ± 0.4	1.29 ± 0.02×10^{-1}	1.22 ± 0.02
	M	ST	6	7	0.24 ± 0.10	199 ± 3	6 ± 0.7	2.68 ± 1.48×10^{-1}	0.22 ± 0.02
		HD	6	7	0.63 ± 0.02	156 ± 0.8	6 ± 0.2	0.26 ± 0.02×10^{-1}	0.22 ± 0.02
		LD	6	7	3.99 ± 0.35	33 ± 0.5	72 ± 0.4	2.35 ± 0.10×10^{-1}	2.60 ± 0.10
	O	ST	6	0	0.21 ± 0.01	167 ± 2	2 ± 0.5	0.25 ± 0.02×10^{-1}	0.19 ± 0.02
		HD	6	0	0.57 ± 0.03	127 ± 3	8 ± 3	6.04 ± 0.52×10^{-1}	0.63 ± 0.13
		LD	6	0	6.91 ± 0.96	49 ± 0.8	62 ± 0.7	1.80 ± 0.08×10^{-1}	2.02 ± 0.07

June	P	ST	18	20	4.64 ± 1.30	318 ± 6	8 ± 0.4	0.30 ± 0.02×10^{-1}	0.30 ± 0.13
		HD	18	20	10.44 ± 3.20	228 ± 0.5	0.3 ± 0.3	0.24 ± 0.03×10^{-1}	0.09 ± 0.03
		LD	18	20	4.46 ± 2.03	44 ± 0.3	67 ± 0.3	1.22 ± 0.02×10^{-1}	1.11 ± 0.03
	M	ST	18	10	4.71 ± 0.69	188 ± 9	15 ± 3	0.63 ± 0.19×10^{-1}	0.84 ± 0.31
		HD	18	10	0.80 ± 0.39	141 ± 17	20 ± 5	1.60 ± 1.22×10^{-1}	0.45 ± 0.11
		LD	18	10	1.59 ± 0.62	33 ± 1	74 ± 1	2.25 ± 0.13×10^{-1}	2.62 ± 0.10
	O	ST	18	1	4.70 ± 2.71	164 ± 15	15 ± 5	0.66 ± 0.22×10^{-1}	0.60 ± 0.27
		HD	18	1	0.74 ± 0.05	131 ± 2	3 ± 2	0.35 ± 0.08×10^{-1}	0.26 ± 0.11
		LD	18	1	6.91 ± 0.61	54 ± 6	58 ± 5	1.96 ± 0.11×10^{-1}	2.06 ± 0.10

Table 3.2: Overview of the four encountered functional groups of macrofauna, with their constituting species. All information concerning functional groups is based on Queirós et al. (2013), except for *Cyathura carinata* (Ólafsson and Persson 1986), *Marenzelleria viridis* (Quintana et al. 2007) and Chironomidae sp. (Lagauzère et al. 2009). Species with multiple functionalities were assigned to each group separately.

Functional group	Species
Surficial modifiers	Bivalvia
	Cerastoderma edule
	Limecola balthica
	Scrobicularia plana

	Crustacea
	Bathyporeia pilosa
	Corophium arenarium
	Corophium volutator
	Cyathura carinata
	Gastropoda
	Peringia ulvae
	Lacuna vincta
	Retusa obtusa
	Polychaeta
	Aphelochaeta marioni
	Marenzelleria viridis
Biodiffusors	Insecta
	Chironomidae sp.
	Oligochaeta spp.
	Polychaeta
	Eteone longa
	Hediste diversicolor
	Phyllodoce maculata
Upward conveyors	Polychaeta
	Arenicola marina
	Capitella capitata
	Heteromastus filiformis
	Polydora cornuta
	Pygospio elegans

	Spio sp.
	Streblospio benedicti
Downward conveyors	Polychaeta
	Polydora cornuta
	Pygospio elegans
	Spio sp.
	Streblospio benedicti

Table 3.3: Mean densities (in individuals m^{-2}) ± standard error for *Aphelochaeta marioni*, *Arenicola marina*, *Bathyporeia pilosa*, *Capitella capitata*, *Cerastoderma edule*, *Chironomidae sp.*, *Corophium arenarium*, *Corophium volutator*, *Cyathura carinata*. *Eteone longa*, *Hediste diversicolor*, *Heteromastus filiformis*, *Lacuna vincta*, *Limecola balthica*, *Marenzelleria viridis*, *Oligochaeta spp.*, *Peringia ulvae*, *Phyllodoce maculata, Polydora cornuta*, *Pygospio elegans*, *Retusa obtusa*, *Scrobicularia plana*, *Spio* sp. and *Streblospio benedicti*. P = polyhaline, M = mesohaline, O = oligohaline, ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal.

			A. marioni	*A. marina*	*B.pilosa*	*C. capitata*	*C. edule*	Chironomidae sp.	*C. arenarium*	*C. volutator*	*C. carinata*
September	P	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9 ± 9	9 ± 9	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	M	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	64 ± 64	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	95 ± 61	0 ± 0	0 ± 0	0 ± 0	414 ± 300	0 ± 0
	O	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Marc December	P	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	432 ± 116	0 ± 0	35 ± 20	0 ± 0	203 ± 67	0 ± 0	18 ± 10
		LD	127 ± 52	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	M	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	95 ± 61	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	32 ± 32	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	732 ± 167	0 ± 0
	O	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	1811 ± 858	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

June		HD	0 ± 0	0 ± 0	1640 ± 356	0 ± 0	0 ± 0	0 ± 0	1032 ± 192	150 ± 53	71 ± 20
		LD	1305 ± 513	0 ± 0	0 ± 0	0 ± 0	32 ± 32	0 ± 0	0 ± 0	64 ± 37	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	M	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	32 ± 32
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	O	HD	0 ± 0	0 ± 0	9 ± 9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	32 ± 32	0 ± 0
	P	HD	0 ± 0	12 ± 12	7654 ± 817	0 ± 0	12 ± 12	12 ± 12	165 ± 51	12 ± 12	0 ± 0
		LD	350 ± 159	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	M	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	127 ± 90	0 ± 0	0 ± 0	0 ± 0	0 ± 0	4106 ± 354	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	O	HD	0 ± 0	0 ± 0	18 ± 10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	32 ± 32	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

			E. longa	*H. diversicolor*	*H. filiformis*	*L. vincta*	*L. balthica*	*M. viridis*	Oligochaeta spp.	*P. ulvae*	*P. maculata*
September		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	P	HD	194 ± 80	44 ± 33	0 ± 0	97 ± 49	1649 ± 1041	0 ± 0	97 ± 51	18 ± 18	0 ± 0
		LD	0 ± 0	541 ± 223	32 ± 32	0 ± 0	159 ± 121	0 ± 0	12827 ± 3746	541 ± 131	0 ± 0
	M	ST	0 ± 0	32 ± 32	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	32 ± 32	255 ± 180	0 ± 0	0 ± 0	0 ± 0	191 ± 82	0 ± 0	0 ± 0

		LD	0 ± 0	509 ± 333	0 ± 0	64 ± 37	0 ± 0	0 ± 0	2324 ± 607	32 ± 32	0 ± 0
	O	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	24 ± 24	0 ± 0	12 ± 12
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5921 ± 973	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	P	HD	247 ± 59	97 ± 30	0 ± 0	0 ± 0	1393 ± 297	0 ± 0	282 ± 50	35 ± 0	0 ± 0
		LD	0 ± 0	64 ± 64	32 ± 32	0 ± 0	127 ± 90	0 ± 0	5316 ± 1575	668 ± 197	0 ± 0
		ST	0 ± 0	0 ± 0	64 ± 37	0 ± 0	64 ± 37	0 ± 0	64 ± 37	0 ± 0	0 ± 0
	M	HD	0 ± 0	0 ± 0	64 ± 64	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	668 ± 121	0 ± 0	0 ± 0	64 ± 37	0 ± 0	828 ± 305	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
December	O	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	24 ± 24	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3247 ± 642	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	64 ± 37	0 ± 0	0 ± 0
	P	HD	9 ± 9	9 ± 9	53 ± 23	0 ± 0	88 ± 18	0 ± 0	203 ± 122	18 ± 18	0 ± 0
		LD	0 ± 0	350 ± 121	64 ± 37	0 ± 0	828 ± 133	0 ± 0	12064 ± 2201	923 ± 240	0 ± 0
		ST	0 ± 0	0 ± 0	605 ± 210	0 ± 0	0 ± 0	0 ± 0	255 ± 138	0 ± 0	0 ± 0
	M	HD	0 ± 0	0 ± 0	64 ± 37	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	732 ± 197	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3342 ± 844	0 ± 0	0 ± 0
		ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	64 ± 37	0 ± 0	0 ± 0
	O	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9 ± 9	53 ± 23	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16457 ± 1183	0 ± 0	0 ± 0
		ST	32 ± 32	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
March	P	HD	176 ± 41	35 ± 20	12 ± 12	24 ± 24	1305 ± 108	0 ± 0	106 ± 41	12 ± 12	0 ± 0
		LD	0 ± 0	32 ± 32	32 ± 32	32 ± 32	286 ± 61	0 ± 0	7066 ± 1356	732 ± 109	0 ± 0
	M	ST	0 ± 0	0 ± 0	64 ± 37	0 ± 0	32 ± 32	0 ± 0	0 ± 0	0 ± 0	0 ± 0
June											

	HD	0 ± 0	64 ± 37	32 ± 32	0 ± 0	32 ± 32	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	LD	0 ± 0	987 ± 217	0 ± 0	0 ± 0	64 ± 37	0 ± 0	1878 ± 551	32 ± 32	0 ± 0
	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	64 ± 64	0 ± 0	0 ± 0
O	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	35 ± 20	53 ± 10	0 ± 0	0 ± 0
	LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	8053 ± 761	0 ± 0	0 ± 0

			P. cornuta	*P. elegans*	*R. obtusa*	*S. plana*	*Spio* sp.	*S. benedicti*
September	P	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	95 ± 32	32 ± 32	0 ± 0	0 ± 0
	M	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	32 ± 32	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	O	HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
December	P	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	9 ± 9	0 ± 0	18 ± 18	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	159 ± 32	0 ± 0	0 ± 0	0 ± 0
	M	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	O	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
March	P	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	18 ± 10	9 ± 9	0 ± 0	0 ± 0	9 ± 9
		LD	0 ± 0	0 ± 0	414 ± 141	32 ± 32	32 ± 32	255 ± 116
	M	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	32 ± 32	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	O	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
June	P	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	12 ± 12	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	286 ± 95	0 ± 0	32 ± 32	0 ± 0
	M	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	O	ST	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		LD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 3.4: Overview of the mean values for all biotic variables ± standard errors, including the benthic processes. P = polyhaline, M = mesohaline, O = oligohaline. ST = subtidal, HD = high-dynamic intertidal, LD = low-dynamic intertidal. Total density is expressed in individuals m^{-2}, total AFDW in g m^{-2}. Particle reworking rate (in cm^2 d^{-1}), Q: bio-irrigation rate in (ml min^{-1} m^{-2}). SM: densities of surficial modifiers, BD: densities of biodiffusors, UC: densities of upward conveyors, DC: densities of downward conveyors; all densities in individuals per m^{-2}.

			Total density	Total AFDW	Particle mixing rate	Q	SM	BD	UC	DC
September	P	ST	0 ± 0	0.00 ± 0.00	$9.80 \pm 2.93 \times 10^{-5}$	2.21 ± 0.08	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	2116 ± 1091	2.06 ± 1.12	$5.58 \pm 2.67 \times 10^{-4}$	4.25 ± 2.54	1772 ± 1042	335 ± 73	0 ± 0	9 ± 9
		LD	14260 ± 3947	8.84 ± 2.17	$1.14 \pm 0.46 \times 10^{-2}$	7.61 ± 1.13	828 ± 152	13369 ± 3882	32 ± 32	0 ± 0
	M	ST	64 ± 37	$4.20 \pm 0.00 \times 10^{-3}$	$5.01 \pm 4.46 \times 10^{-4}$	2.52 ± 0.09	0 ± 0	32 ± 32	0 ± 0	0 ± 0
		HD	573 ± 198	1.00 ± 0.75	$3.91 \pm 3.11 \times 10^{-3}$	3.54 ± 0.88	32 ± 32	223 ± 80	318 ± 160	0 ± 0
		LD	3438 ± 1122	2.04 ± 1.16	$1.47 \pm 0.86 \times 10^{-2}$	6.76 ± 2.08	509 ± 260	2833 ± 938	95 ± 61	0 ± 0
	O	HD	35 ± 35	$6.31 \pm 6.31 \times 10^{-3}$	$1.15 \pm 0.35 \times 10^{-3}$	1.47 ± 1.00	0 ± 0	35 ± 35	0 ± 0	0 ± 0
		LD	5921 ± 973	0.55 ± 0.10	$5.98 \pm 3.70 \times 10^{-5}$	4.15 ± 0.13	0 ± 0	5921 ± 973	0 ± 0	0 ± 0
December	P	ST	0 ± 0	0.00 ± 0.00	$2.86 \pm 1.34 \times 10^{-5}$	2.87 ± 0.06	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	2769 ± 304	3.86 ± 1.29	$1.67 \pm 0.91 \times 10^{-2}$	10.04 ± 1.25	2134 ± 300	626 ± 44	9 ± 9	9 ± 9
		LD	6494 ± 1718	3.66 ± 0.83	$6.10 \pm 1.76 \times 10^{-5}$	4.76 ± 0.79	1082 ± 287	5379 ± 1539	32 ± 32	0 ± 0
	M	ST	286 ± 141	2.25 ± 1.49	$6.08 \pm 3.31 \times 10^{-4}$	2.99 ± 0.26	159 ± 80	64 ± 37	64 ± 37	0 ± 0
		HD	95 ± 61	$3.22 \pm 2.92 \times 10^{-2}$	$9.81 \pm 6.15 \times 10^{-3}$	2.11 ± 0.24	32 ± 32	0 ± 0	64 ± 64	0 ± 0
		LD	2292 ± 462	3.22 ± 0.96	$3.90 \pm 2.32 \times 10^{-2}$	7.17 ± 0.84	796 ± 141	1496 ± 394	0 ± 0	0 ± 0
	O	ST	0 ± 0	0.00 ± 0.00	$4.51 \pm 1.49 \times 10^{-4}$	2.35 ± 0.08	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		HD	1834 ± 847	0.16 ± 0.05	$4.34 \pm 2.64 \times 10^{-3}$	1.35 ± 0.51	1811 ± 858	24 ± 24	0 ± 0	0 ± 0
		LD	3247 ± 642	0.21 ± 0.03	$1.43 \pm 0.96 \times 10^{-2}$	2.47 ± 0.13	0 ± 0	3247 ± 642	0 ± 0	0 ± 0
March	P	ST	95 ± 32	$6.66 \pm 4.66 \times 10^{-3}$	$9.27 \pm 6.04 \times 10^{-4}$	4.45 ± 0.53	0 ± 0	64 ± 37	0 ± 0	0 ± 0
		HD	3315 ± 648	1.31 ± 0.42	$7.17 \pm 1.91 \times 10^{-3}$	7.00 ± 1.52	3016 ± 558	220 ± 121	79 ± 17	26 ± 17
		LD	16297 ± 3164	12.59 ± 5.34	$2.24 \pm 0.66 \times 10^{-3}$	6.08 ± 0.52	3533 ± 870	12414 ± 2213	350 ± 141	286 ± 109
	M	ST	859 ± 257	0.12 ± 0.04	$3.10 \pm 2.86 \times 10^{-3}$	4.15 ± 0.38	0 ± 0	255 ± 138	605 ± 210	0 ± 0

		HD	127 ± 90	1.01 ± 0.58×10^{-2}	2.69 ± 1.53×10^{-4}	4.40 ± 0.19	32 ± 32	0 ± 0	95 ± 61	32 ± 32
		LD	4138 ± 942	2.07 ± 0.46	3.42 ± 1.94×10^{-3}	8.34 ± 0.94	64 ± 37	4074 ± 937	0 ± 0	0 ± 0
		ST	64 ± 37	4.67 ± 3.20×10^{-3}	1.35 ± 0.44×10^{-4}		0 ± 0	64 ± 37	0 ± 0	0 ± 0
	O	HD	71 ± 29	1.13 ± 0.63×10^{-2}	2.55 ± 2.37×10^{-3}		9 ± 9	62 ± 22	0 ± 0	0 ± 0
		LD	16457 ± 1183	1.00 ± 0.06	5.58 ± 2.11×10^{-3}		0 ± 0	16457 ± 1183	0 ± 0	0 ± 0
		ST	64 ± 64	1.86 ± 1.86×10^{-2}	1.50 ± 0.24×10^{-2}	3.68 ± 1.72	32 ± 32	32 ± 32	0 ± 0	0 ± 0
	P	HD	9546 ± 755	4.14 ± 1.53	2.59 ± 0.76×10^{-2}	3.03 ± 0.72	9182 ± 719	317 ± 73	35 ± 0	24 ± 12
		LD	9008 ± 1412	7.91 ± 1.14	2.12 ± 0.67×10^{-3}	5.26 ± 0.71	1687 ± 95	7098 ± 1326	64 ± 64	32 ± 32
		ST	95 ± 32	0.96 ± 0.94	2.75 ± 1.21×10^{-3}	4.17 ± 0.55	32 ± 32	0 ± 0	64 ± 37	0 ± 0
	M	HD	127 ± 52	0.56 ± 0.45	1.99 ± 0.63×10^{-4}	15.02 ± 1.28	32 ± 32	64 ± 37	0 ± 0	0 ± 0
		LD	7194 ± 907	3.56 ± 0.65	8.64 ± 3.67×10^{-3}	1.93 ± 2.56	4329 ± 425	2865 ± 521	0 ± 0	0 ± 0
		ST	95 ± 61	2.78 ± 2.11×10^{-2}	3.41 ± 0.71×10^{-4}	4.08 ± 0.16	0 ± 0	64 ± 64	0 ± 0	0 ± 0
	O	HD	106 ± 20	2.39 ± 1.12×10^{-2}	4.67 ± 2.91×10^{-3}	2.56 ± 0.79	18 ± 10	88 ± 10	0 ± 0	0 ± 0
June		LD	8085 ± 738	1.05 ± 1.13	2.66 ± 1.42×10^{-3}	4.20 ± 0.78	32 ± 32	8053 ± 761	0 ± 0	0 ± 0

Table 3.5: Overview of the mean values for the fluxes of oxygen and nutrients ± standard errors. All values are in mmol m^{-2} d^{-1}.

			Oxygen flux	Nitrate flux	Nitrite flux	Ammonia flux	Phosphate flux
September	P	ST	-26.77 ± 3.00	0.41 ± 0.32	1.91 ± 1.84×10^{-2}	0.53 ± 0.61	0.74 ± 0.43
		HD	-48.96 ± 10.51	0.07 ± 0.06	0.14 ± 2.57×10^{-3}	16.86 ± 11.01	1.76 ± 1.08
		LD	-55.87 ± 12.72	-0.12 ± 0.05	0.95 ± 1.14×10^{-2}	15.15 ± 7.69	0.66 ± 1.39
	M	ST	-39.25 ± 4.16	-0.27 ± 0.03	0.19 ± 0.05	-5.53 ± 3.80	-1.16 ± 1.44
		HD	-33.71 ± 6.75	0.17 ± 0.03	3.16 ± 0.80×10^{-2}	3.35 ± 1.88	1.58 ± 0.49
		LD	-45.10 ± 6.97	0.09 ± 0.10	0.15 ± 0.03	-0.59 ± 5.77	1.49 ± 1.19
	O	HD	-20.59 ± 1.70	1.31 ± 0.81	-7.32 ± 2.13×10^{-2}	7.76 ± 5.64	1.65 ± 1.07
		LD	-40.22 ± 1.50	0.51 ± 0.51	-2.88 ± 4.60×10^{-2}	2.98 ± 0.34	3.76 ± 3.44×10^{-2}
	P	ST	-23.09 ± 3.23	0.10 ± 0.05	8.48 ± 6.36×10^{-3}	-0.39 ± 0.38	-0.64 ± 13.6×10^{-2}
		HD	-39.99 ± 14.81	0.04 ± 0.07	6.86 ± 2.85×10^{-3}	0.20 ± 0.57	8.81 ± 3.00×10^{-2}
		LD	-14.68 ± 4.01	-0.10 ± 1.56×10^{-2}	3.84 ± 0.50×10^{-2}	-0.35 ± 1.77	-0.23 ± 0.13
	M	ST	-24.97 ± 2.63	0.19 ± 0.22	0.18 ± 0.16	-0.42 ± 0.39	0.58 ± 2.10×10^{-1}
		HD	-33.06 ± 9.66	-0.13 ± 0.10	1.15 ± 2.69×10^{-2}	-0.85 ± 0.47	3.52 ± 8.23×10^{-2}
		LD	-29.77 ± 2.40	-0.05 ± 0.12	0.20 ± 2.61×10^{-2}	0.42 ± 0.45	3.44 ± 5.04×10^{-2}
	O	ST	-25.68 ± 3.09	0.43 ± 0.24	0.14 ± 0.22	-1.48 ± 0.52	-0.34 ± 0.24
		HD	-42.17 ± 20.11	0.75 ± 0.51	0.63 ± 1.67×10^{-2}	-5.82 ± 2.71	-1.75 ± 0.45
		LD	-22.55 ± 2.80	0.02 ± 1.57×10^{-1}	-3.55 ± 7.25×10^{-2}	-1.75 ± 0.84	-0.35 ± 0.39
December	P	ST	-29.49 ± 3.74	-0.68 ± 1.18	6.19 ± 7.42×10^{-2}	-1.76 ± 0.88	-0.11 ± 1.40×10^{-1}
		HD	-87.64 ± 4.47	-0.78 ± 0.10	-8.92 ± 1.84×10^{-2}	1.22 ± 1.45	0.26 ± 0.59
		LD	-51.98 ± 3.85	0.16 ± 0.38	1.40 ± 3.70×10^{-2}	-0.75 ± 1.65	0.14 ± 0.38
	M	ST	-23.22 ± 4.19	-0.08 ± 0.37	-2.30 ± 3.84×10^{-2}	-0.89 ± 0.71	-0.32 ± 0.13
		HD	-28.71 ± 5.43	-0.45 ± 0.18	-0.11 ± 0.02	-1.61 ± 1.33	-0.53 ± 0.27
March		LD	-27.89 ± 3.58	4.79 ± 0.80	0.73 ± 0.12	-3.11 ± 1.88	-0.96 ± 0.50

June	O	ST	-20.80 ± 1.14	-1.47 ± 0.50	-0.23 ± 0.06	-3.01 ± 2.33	-0.57 ± 0.43
		HD	-52.70 ± 11.35	-0.43 ± 0.86	$-5.34 \pm 8.61\times10^{-2}$	-2.49 ± 2.14	-0.82 ± 0.52
		LD	-27.70 ± 7.52	-7.84 ± 1.88	-1.09 ± 0.25	-1.74 ± 1.35	-0.50 ± 0.36
	P	ST	-24.44 ± 3.83	0.35 ± 0.29	$3.90 \pm 2.03\times10^{-2}$	-0.17 ± 0.22	0.19 ± 0.57
		HD	-20.51 ± 2.80	0.79 ± 1.65	$0.81 \pm 1.55\times10^{-1}$	0.32 ± 0.69	-0.49 ± 0.94
		LD	-59.96 ± 9.70	3.30 ± 1.31	0.40 ± 0.13	6.84 ± 2.56	0.37 ± 0.85
	M	ST	-25.86 ± 3.31	0.97 ± 0.63	$7.05 \pm 3.75\times10^{-2}$	$3.27 \pm 2.05\times10^{-2}$	0.44 ± 0.22
		HD	-24.17 ± 3.93	1.89 ± 0.55	$0.11 \pm 0.05\times10^{-2}$	0.82 ± 0.44	1.07 ± 0.78
		LD	-44.35 ± 5.23	2.63 ± 1.37	0.25 ± 0.13	2.07 ± 1.68	2.99 ± 2.38
	O	ST	-16.19 ± 3.28	1.02 ± 0.18	$5.17 \pm 0.57\times10^{-2}$	1.79 ± 0.81	0.80 ± 0.84
		HD	-16.67 ± 6.12	0.35 ± 2.14	$0.89 \pm 2.93\times10^{-1}$	0.99 ± 1.79	1.13 ± 2.75
		LD	-36.83 ± 7.56	2.04 ± 1.31	0.16 ± 0.07	0.75 ± 2.00	-0.97 ± 3.40

Figure 3.1: nMDS plots of functional variables, the fluxes of (a) Oxygen (b) Ammonia (c) Nitrite (d) Nitrate.

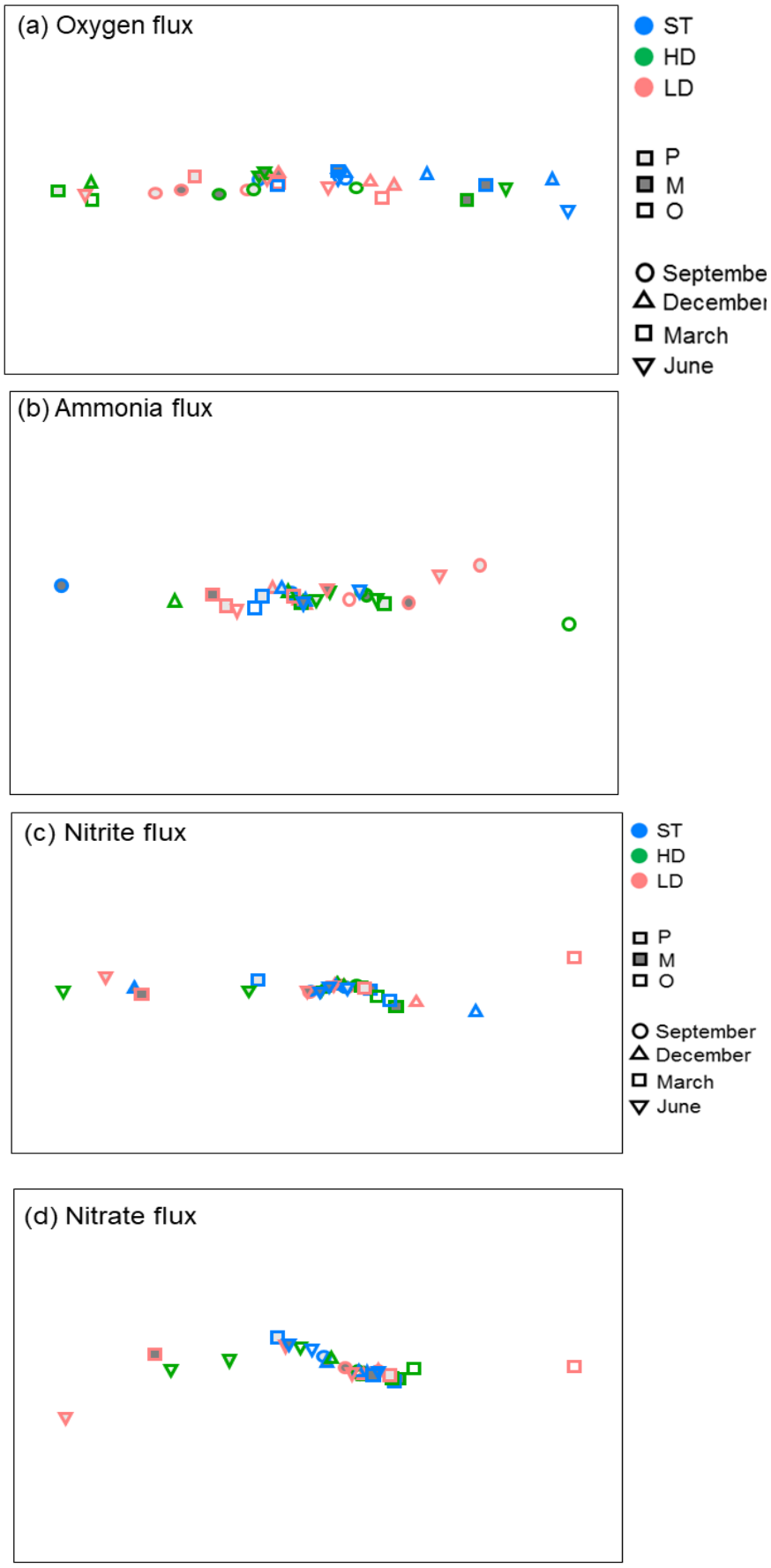
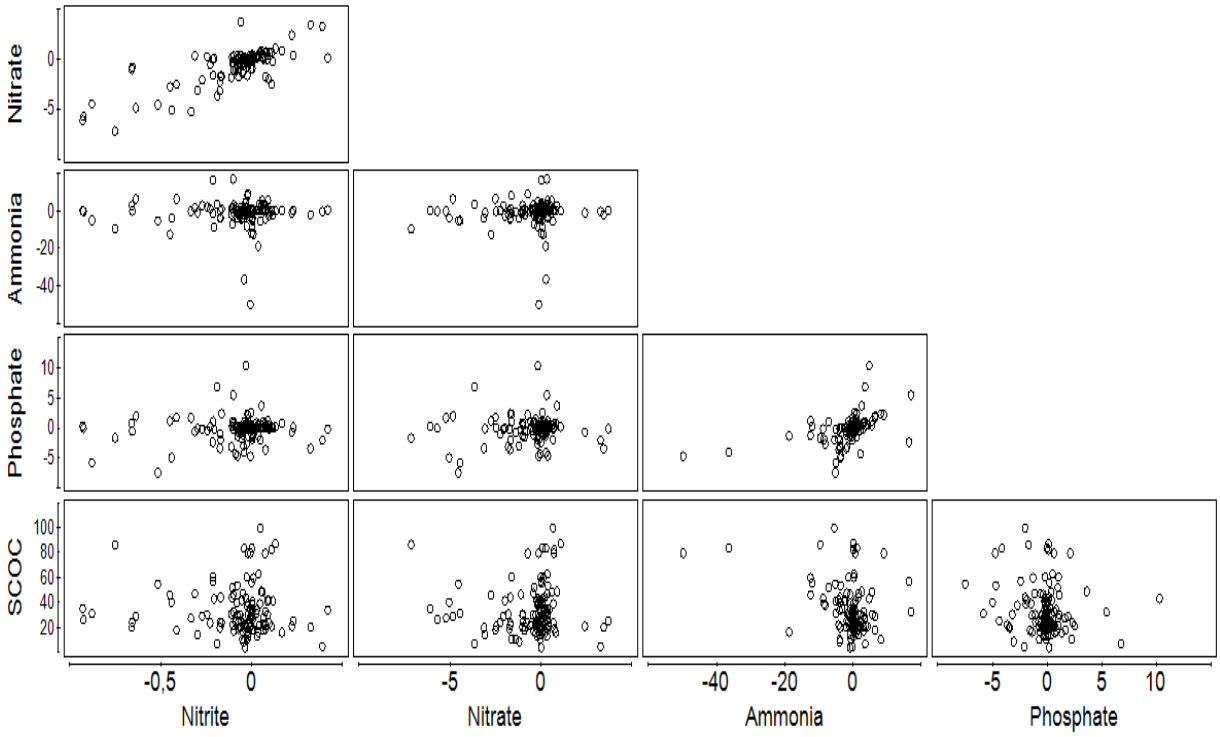


Figure 3.2: Plot representing the correlation coefficients between environmental variables, macrofaunal variables and benthic fluxes for the complete dataset, (a): Draftsman plots showing the correlation between benthic fluxes (b): Correlation among all predictor variables in DitsLM models. S = Density of Surficial modifier; B = Density of Biodiffusor; UC = Density of Upward conveyor; DC = Density of Downward Conveyor; Mudpct = Mud content; TN = Total Nitrogen; MGS = Median Grain Size; TOC = Total Organic Carbon; SAFDW = Biomass of surficial modifier; BAFDW = Biomass of biodiffusor; UCAFDW = Biomass of upward conveyor; DCAFDW = Biomass of downward conveyor; Denstot = Total Density; AFDWtot = Total Biomass.

(a)



(b)

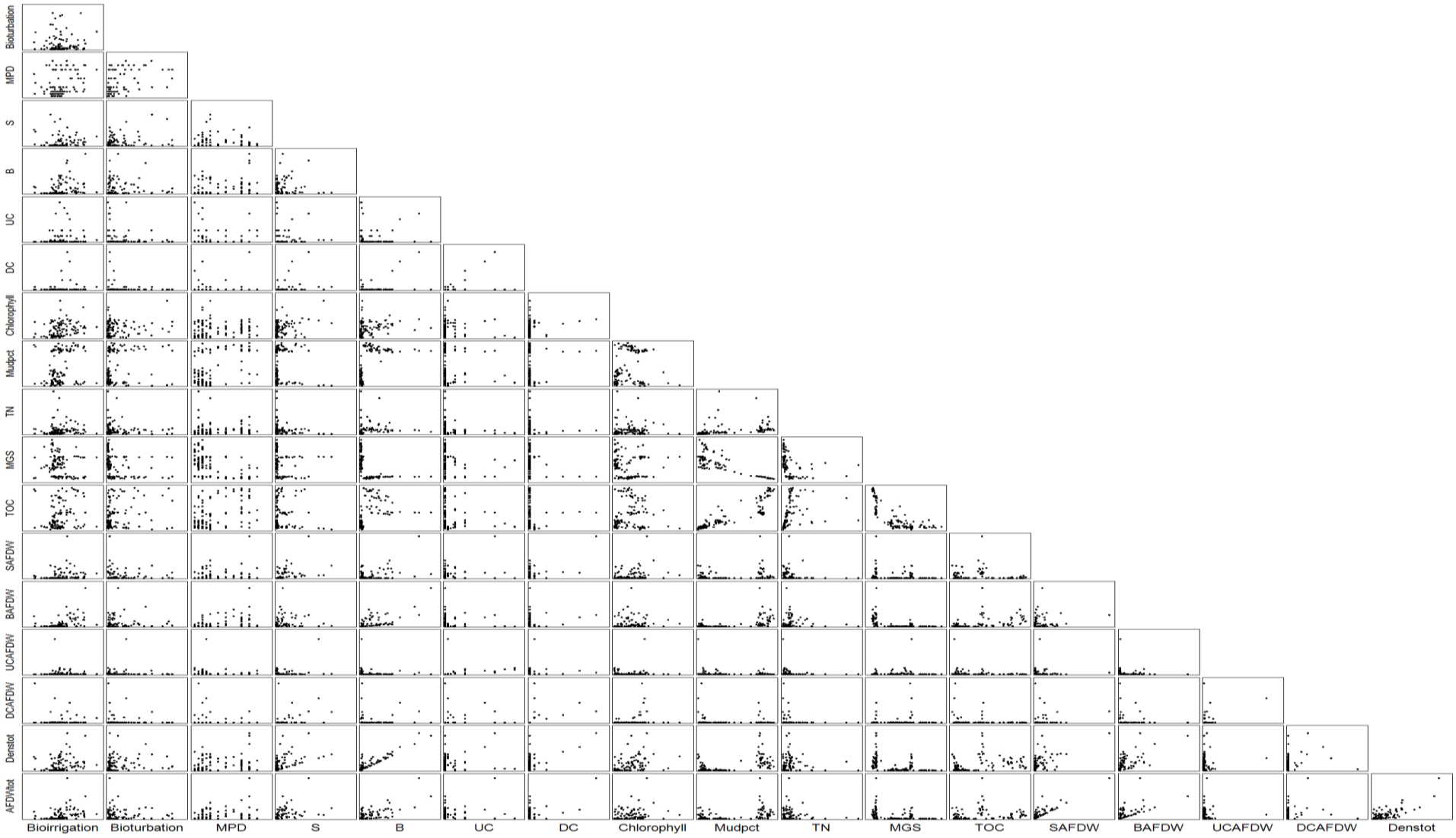
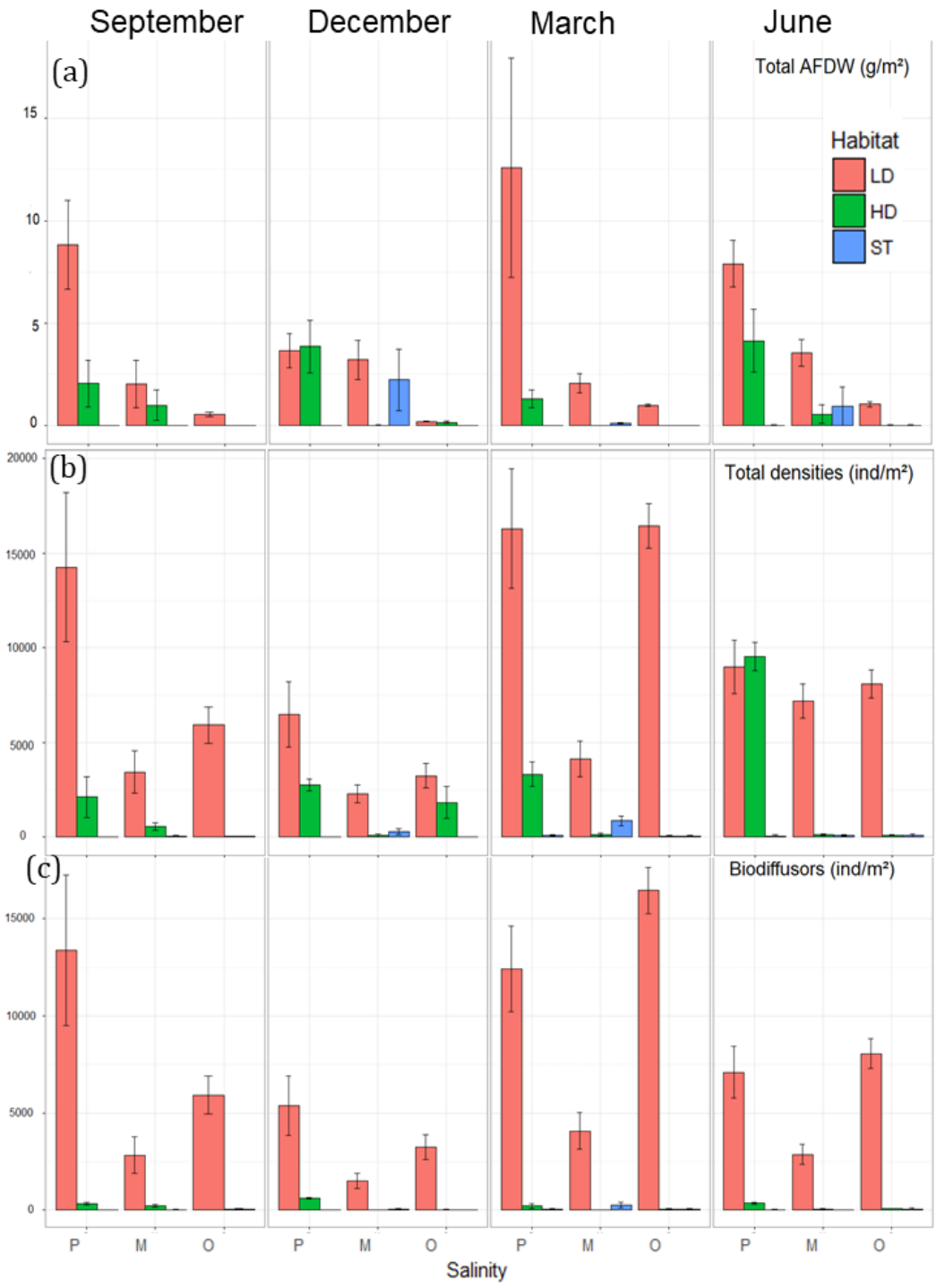

Bioturbation
MPD
S
B
UC
DC
Chlorophyll
Mudpct
TN
MGS
TOC
SAFDW
BAFDW
UCAFDW
DCAFDW
Denstot
AFDWtot
Bioirrigation
Bioturbation
MPD
S
B
UC
DC
Chlorophyll
Mudpct
TN
MGS
TOC
SAFDW
BAFDW
UCAFDW
DCAFDW
Denstot

Figure 3.3: Overview of the macrofaunal descriptors (a) total ash-free dry weights (AFDW), (b) total density, (c) density of biodiffusors, (d) density of surficial modifiers, (e) density of upward conveyors and (f) density of downward conveyors. Error bars represent the deviation of standard errors from the mean. LD = low-dynamic intertidal, HD = high-dynamic intertidal, ST=subtidal. P = polyhaline, M = mesohaline, O = Oligohaline.



Supplementary material for chapter 4

Figure 4.1: Photos of experimental set-up and chronological overview of the experimental activities.

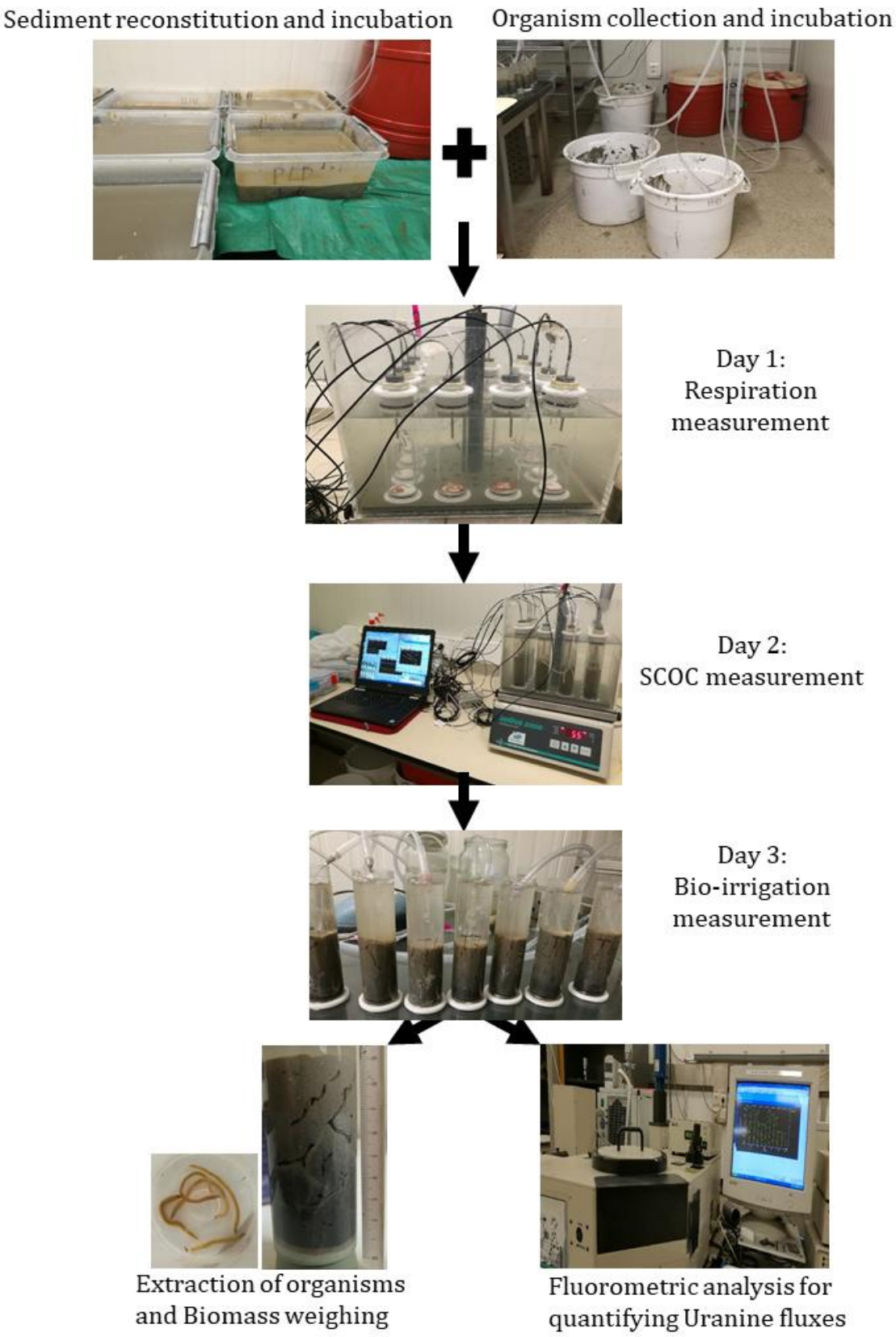
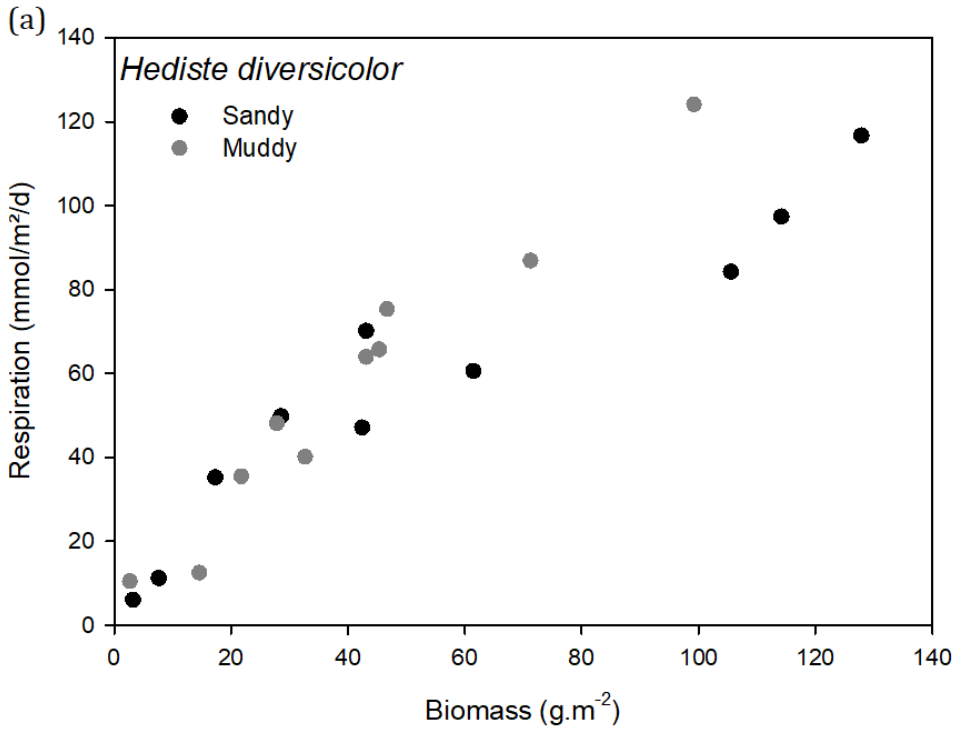


Figure 4.2: Scatter plots of respiration (mmol m^{-2} d^{-1}) measured in each microcosm fill with *in situ* seawater and animal biomass (AFDW) for (a) *H. diversicolor* and (b) *L. balthica*.



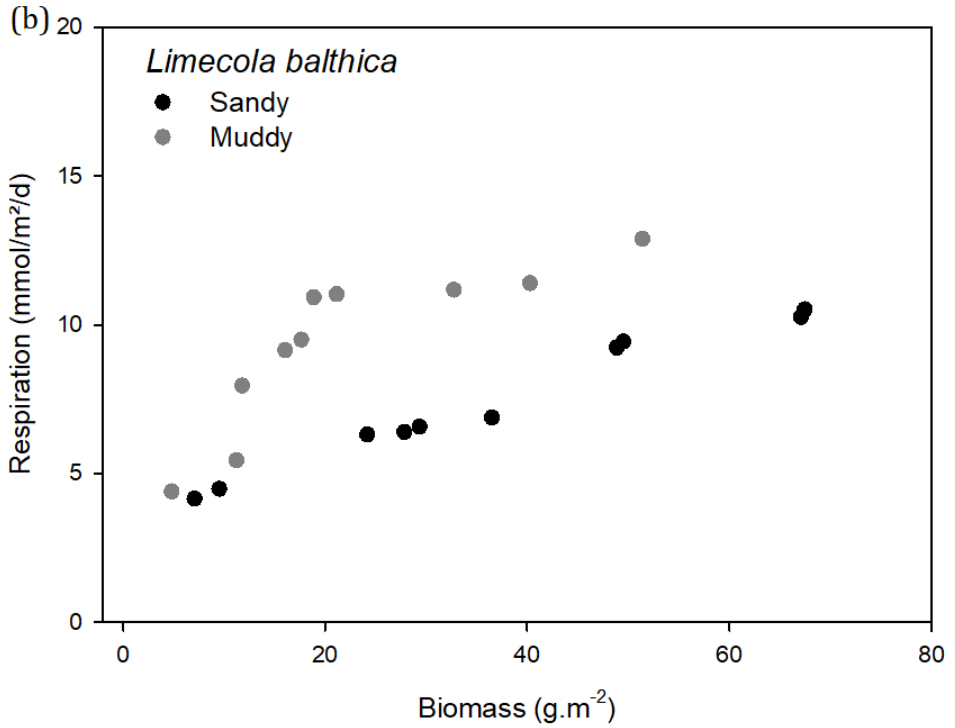


Figure 4.3: Sediment characteristics at polyhaline high – dynamic (PHD) and polyhaline low – dynamic (PLD) habitats, derived from the analysis results of baseline survey in year 2015/2016 (PhD thesis Chapter 2): (a) Oxygen penetration depth (in mm), (b) Total organic carbon (in %), (c) Permeability. The incubated cores with 10cm sediments were sliced in 0.5 cm for the first four slices, and 1 cm thick slices further down. 2 ml from each slice for analysis of total organic carbon and a final 30 ml from the homogenised entire sample for analysis of sediment permeability.

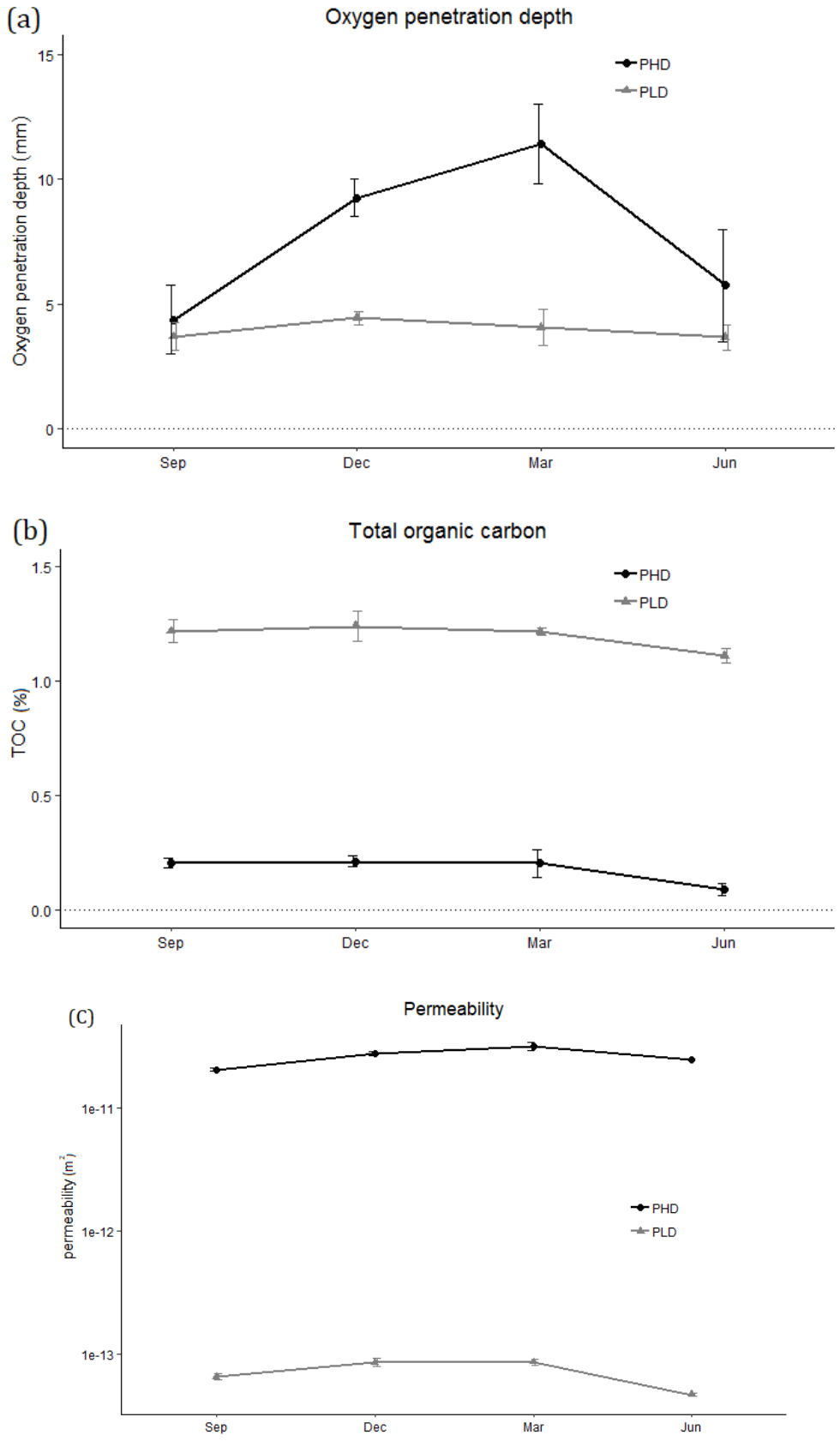


Table 4.1: To exclude the organisms biovolume from the water volume, the calibration relationships established between Biomass (AFDW, in g) and biovolume (in ml) of target species (a) *H. diversicolor* and

(b) *L. balthica*. Biovolume was determined from volume displaced when experimented individuals were immersed in a known volume of water in a graduated cylinder (Persoone, 1971).

(a) H. diversicolor

Habitat	Biovolume (ml)	Length (mm)	Width (mm)	Wet weight (g)	Dry weight (g)	AFDW (g)
PHD	0.32	92.14	2.85	0.35	0.04	0.03
PHD	0.3	83.46	2.32	0.33	0.04	0.03
PHD	0.65	105.48	3.51	0.79	0.07	0.07
PHD	0.07	53.81	2.31	0.08	0.01	0.01
PHD	0.06	18.12	2.59	0.04	0.004	0.003
PHD	0.19	49.04	2.53	0.19	0.03	0.02
PHD	0.1	13.02	3.97	0.09	0.01	0.01
PHD	0.15	33.75	3.49	0.11	0.02	0.01
PHD	0.45	79.22	3.55	0.50	0.06	0.04
PHD	0.54	117.25	3.02	0.72	0.07	0.05
PHD	0.6	87.66	3.51	0.76	0.07	0.05
PHD	0.4	107.38	2.89	0.45	0.05	0.04
PLD	0.45	113.67	4.35	0.65	0.07	0.06
PLD	0.49	97.04	3.14	0.43	0.06	0.06
PLD	0.74	94.34	3.74	0.66	0.09	0.08
PLD	0.17	42.11	3.59	0.10	0.02	0.01
PLD	0.45	81.74	3.36	0.40	0.05	0.04
PLD	0.1	32.29	2.49	0.09	0.01	0.01
PLD	0.1	41.54	2.06	0.08	0.01	0.01
PLD	0.08	22.63	1.89	0.04	0.004	0.003
PLD	0.25	60.91	2.05	0.19	0.03	0.02
PLD	0.35	123.71	2.06	0.29	0.05	0.04
PLD	0.6	96.84	3.52	0.52	0.06	0.04

(b) L. balthica

Habitat	Biovolume (ml)	Length (mm)	Width (mm)	Wet weight (g)	Dry weight (g)	AFDW (g)
PHD	0.1	6.5	8.22	0.09	0.04	0.01
PHD	0.2	8.37	10.96	0.21	0.10	0.02
PHD	0.2	8.94	11.29	0.25	0.13	0.02
PHD	0.2	9.46	12.22	0.30	0.16	0.02
PHD	0.46	11.07	13.97	0.53	0.27	0.05
PHD	0.3	10.31	13.4	0.44	0.23	0.04
PHD	0.35	10.49	13.36	0.47	0.26	0.05
PHD	0.23	9.14	12.07	0.28	0.12	0.02
PHD	0.3	10.35	13.15	0.40	0.19	0.02
PHD	0.5	11.42	14.72	0.71	0.38	0.07
PHD	0.35	10.26	13.33	0.53	0.29	0.05
PLD	0.01	4.96	6.23	0.04	0.01	0.002
PLD	0.09	6.45	8.09	0.11	0.05	0.01
PLD	0.12	8.81	10.73	0.28	0.14	0.02
PLD	0.2	8.83	11.32	0.37	0.19	0.04
PLD	0.23	9.43	11.78	0.07	0.03	0.03
PLD	0.35	11.24	13.8	0.33	0.17	0.04
PLD	0.3	10.62	13.48	0.39	0.19	0.03
PLD	0.35	10.81	13.1	0.29	0.14	0.03
PLD	0.2	9.13	11.68	0.31	0.15	0.03
PLD	0.3	10.21	12.9	0.40	0.21	0.04
PLD	0.25	11.44	15.21	0.42	0.31	0.04
PLD	0.2	10.62	13.47	0.63	0.35	0.03

Table 4.2: O_2 uptake in sea water and sediment (mmol m^{-2} d^{-1}) and Uranine flux (L d m^{-2}) measured in the control cores (abiotic control) in four experiment schemes.

Experiment scheme	O_2 uptake in sea water (mmol m^{-2} d^{-1})		O_2 uptake in sediment (mmol m^{-2} d^{-1})		Uranine flux (L m^{-2} d^{-1})	
Species	Muddy	Sandy	Muddy	Sandy	Muddy	Sandy
L. balthica	1.275	1.279	10.359	2.179	12.762	3.437
H. diversicolor	0.813	1.28	7.159	1.712	12.528	2.023

Table 4.3: Test of ANCOVA assumptions equality of error variances by Levene's Test. Uranine fluxes mediated by *L. balthica* were log transformed.

Species	Dependent Variable	F	df1	df2	Sig.
H. diversicolor	Faunal-mediated O_2 uptake	0.017	1	18	0.899
	Faunal-mediated Uranine flux	0.245	1	18	0.627
L. balthica	Faunal-mediated O_2 uptake	1.833	1	18	0.193
	Faunal-mediated Uranine flux	0.949	1	18	0.343

Table 4.4: A summary of multiple regression models (dependent variable ~ biomass + sediment + biomass * sediment). The assumption of homogeneity of regression slopes tested in species subsets (a) *H. diversicolor* and (b) *L. balthica* with multiple linear regression with biomass (continuous), sediment (categorical) and their interactions as explanatory variables to predict the measured processes (i.e. Dependent Variable Faunal-mediated O2 uptake and Faunal-mediated Uranine flux). The factor sediment and covariate biomass do not interact (p>0.05).

(a) *H. diversicolor*

Faunal-mediated O_2 uptake ~ biomass + sediment + sediment * biomass

R^2 = 0.86 (Adjusted R^2 = 0.84)

	Type III Sum of Squares	Mean Square	F	Sig.
Corrected Model	36669.09	12223.03	33.21	<0.001

Intercept	4252.63	4252.63	11.55	<0.001
sediment	0.23	0.23	0.003	0.98
biomass	34834.15	34834.15	94.63	<0.001
sediment * biomass	8.30	8.30	0.02	0.88
Error	5889.52	368.10		
Total	197874.49			
Corrected Total	42558.61			

Faunal-mediated Uranine Flux~ biomass + sediment + sediment * biomass

R^2 = 0.7 (Adjusted R^2 = 0.64)

Corrected Model	308.26	102.75	12.21	<0.001
Intercept	1067.35	1067.35	126.84	<0.001
sediment	13.37	13.37	1.59	0.23
biomass	225.78	225.78	26.83	<0.001
sediment * biomass	14.01	14.01	1.67	0.22
Error	134.64	8.42		
Total	6476.21			
Corrected Total	442.90			

(b) *L. balthica*

Faunal-mediated O_2 uptake ~ biomass + sediment + sediment * biomass

R^2 = 0.87 (Adjusted R^2 = 0.85)

	Type III Sum of Squares	Mean Square	F	Sig.
Corrected Model	1008.49	336.16	35.89	<0.001
Intercept	58.73	58.73	6.27	0.02
sediment	42.58	42.58	4.55	0.05
biomass	897.48	897.48	95.82	<0.001
sediment * biomass	17.25	17.25	1.84	0.19
Error	149.86	9.37		
Total	5885.96			

Corrected Total	1158.35			

Faunal-mediated Uranine Flux~ biomass + sediment + sediment * biomass

R^2 = 0.76 (Adjusted R^2 = 71)

Corrected Model	1044.22	348.07	16.76	<0.001
Intercept	247.19	247.19	11.90	<0.001
sediment	6.39	6.39	0.31	0.59
biomass	282.32	282.32	13.59	<0.001
sediment * biomass	40.04	40.04	1.93	0.18
Error	332.33	20.77		
Total	5629.94			
Corrected Total	1376.55			

Supplementary material for chapter 5

Figure 5.1: Maximal biomass (gAFDW m^{-2}) of *H. diversicolor* in the Western Scheldt in (a) 1955, (b) 2010 and the (c) Difference over the time span $\Delta_{2010\text{-}1955}$, estimated from potential niche map of *H. diversicolor* (Quantile regression, tau=0.95).

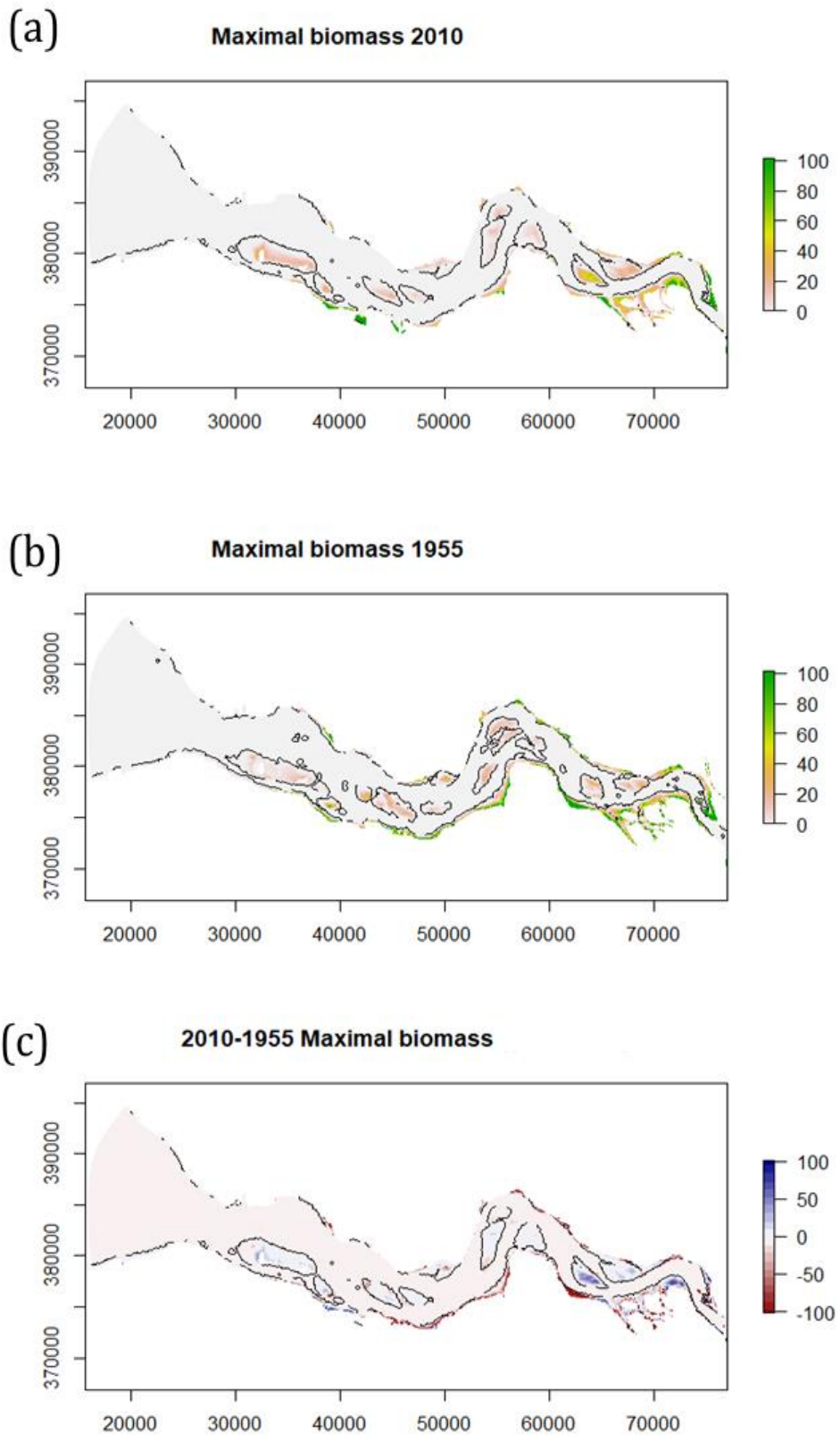


Figure 5.2: A numeric comparison in the realistic domain. Realized biomass of *H. diversicolor* (gAFDW m^{-2}) in the Western Scheldt in (a) 1955, (b) 2010, and (c) Boxplot of the full quantile distribution of *H. diversicolor*, estimated from full quantile of *H. diversicolor* distribution (Quantile regression, tau=0.01-0.99).

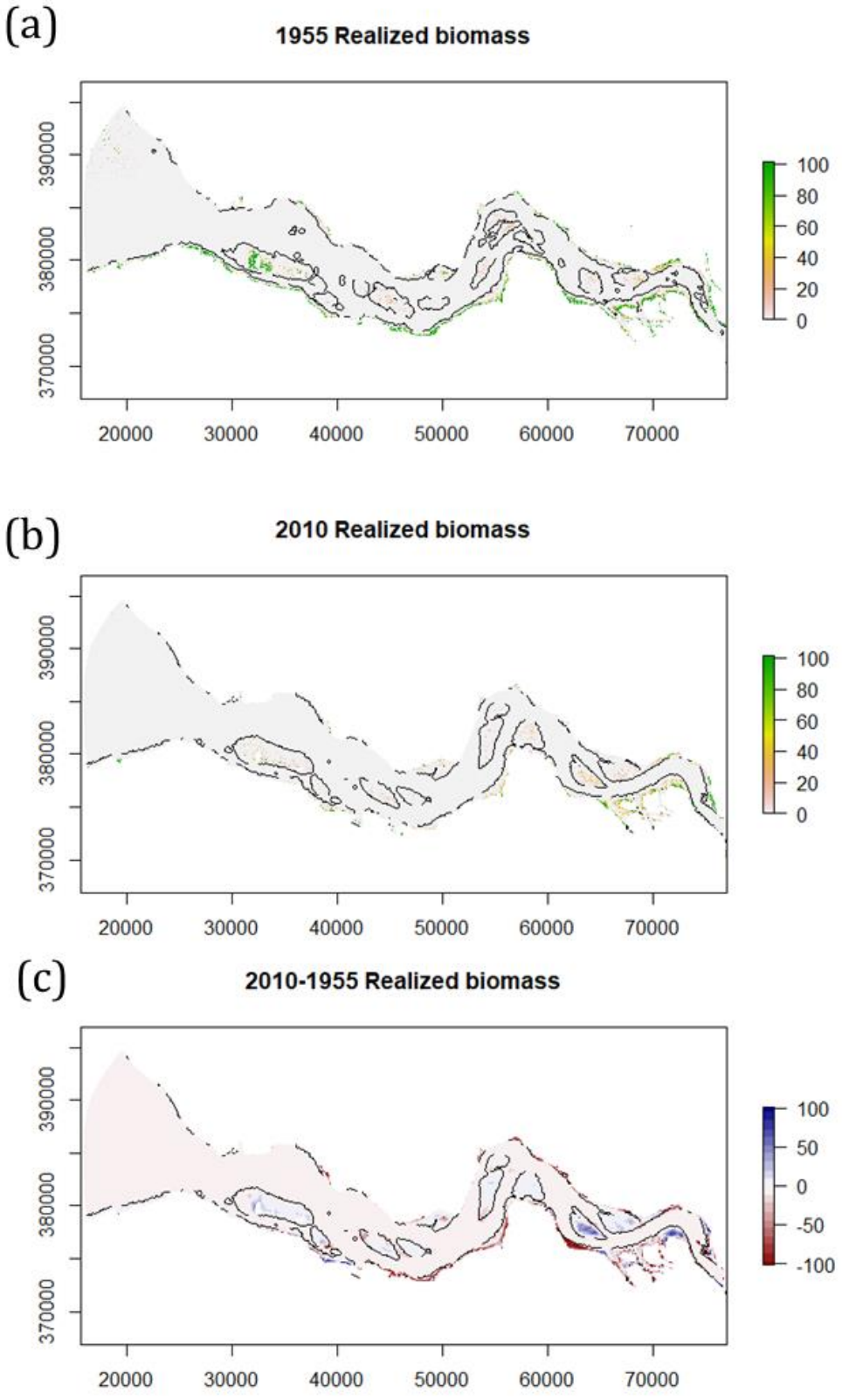


Figure 5.3: A model diagram of species distribution modelling (model equations can be found below in Table 5.1a,b)

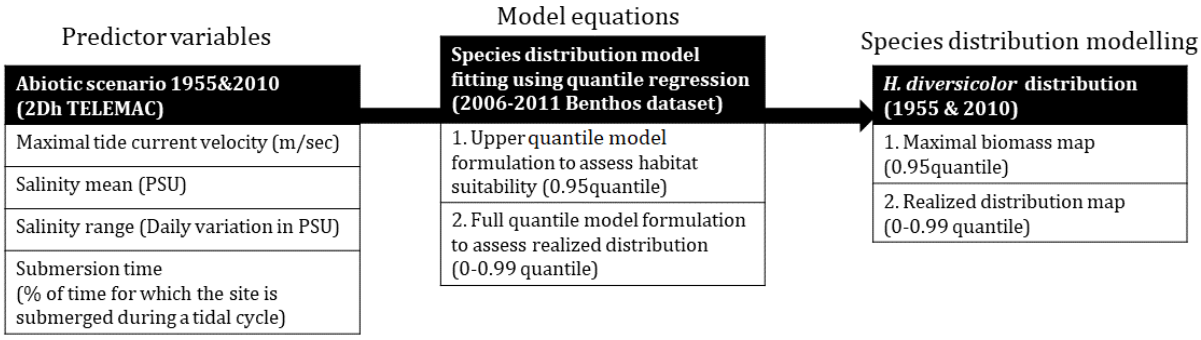


Table 5.1: Summary of the (a) 95th quantile regression model and (b) full quantile model. Standard errors were calculated by an inverted rank test. vel = current velocity; salmean = mean salinity; salrange = daily salinity range; em = inundation time.

(a) Upper quantile model (tau=0.95)	Value	Std. Error	t value	Pr(>|t|)
(Intercept)	10.24	7.24	1.41	0.16
vel	0.86	13.64	0.06	0.95
salmean	0.15	0.29	0.51	0.61
log(salrange)	-5.95	3.75	-1.59	0.11
asin(em)	9.24	12.31	0.75	0.45
vel:salmean	-0.23	0.55	-0.42	0.67
vel:log(salrange)	27.26	7.33	3.72	<0.001
salmean:log(salrange)	0.31	0.15	2.00	0.05
vel:asin(em)	-10.40	12.66	-0.82	0.41
salmean:asin(em)	-0.57	0.49	-1.15	0.25
log(salrange):asin(em)	-1.56	6.44	-0.24	0.81
vel:salmean:log(salrange)	-1.08	0.31	-3.48	<0.001
vel:salmean:asin(em)	0.45	0.50	0.89	0.37
vel:log(salrange):asin(em)	-14.08	6.71	-2.10	0.04
salmean:log(salrange):asin(em)	-0.05	0.26	-0.21	0.84
vel:salmean:log(salrange):asin(em)	0.60	0.28	2.18	0.03

	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=
	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.54	0.59	0.64	0.69	0.74	0.79	0.84	0.89	0.94	0.99
(b) Full quantile model	0	9	8	7	6	5	4	3	2	1	0	9	8	7	6	5	4	3	2	1	0
	4.05	9.64	1.04	1.56	27.6	32.1	43.8	43.7	44.4	44.4	41.5	39.0	35.1	32.2	27.1	19.6	15.2	17.4	20.2		24.2
(Intercept)	E-12	E-11	E-09	E-11	1	0	0	5	3	9	1	1	8	0	9	6	0	0	9	6.83	2
	-	-	-	-	-	-	-	-	-	-	-	-	-	-							-
	1.26	1.08	1.23	1.90	37.2	41.5	52.4	51.2	50.1	47.0	38.7	32.9	23.0	16.9	-	12.2					14.4
vel	E-11	E-10	E-09	E-11	0	6	8	7	3	7	7	4	4	3	6.95	6.14	3	7.56	2.11	9.35	2
	-	-	-	-																	
	2.13	3.11	3.32	5.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
salmean	E-13	E-12	E-11	E-13	0.90	1.03	1.40	1.31	1.30	1.27	1.11	1.00	0.85	0.71	0.50	0.22	0.03	0.09	0.20	0.30	0.52
	-	-	-	-	-	-	-														
	6.67	3.89	3.85	5.95	17.1	17.7	10.8	-	-			10.9	14.1	13.8	14.4	15.8		-	-	-	11.4
log(salrange)	E-12	E-11	E-10	E-12	8	7	3	2.34	1.08	6.20	8.65	5	4	4	4	4	5.71	0.16	5.84	5.99	1
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-		-
	2.29	1.01	9.13	1.11	17.5	20.4	27.8	27.8	28.2	28.3	26.4	24.8	22.4	20.5	17.3	12.5	-	11.0	12.9	10.7	15.3
asin(em)	E-12	E-10	E-10	E-11	8	4	9	5	9	2	2	3	0	0	1	1	9.68	8	1	2	8
	5.16	2.93	3.67	5.94											-	-	-	-	-	-	
vel:salmean	E-13	E-12	E-11	E-13	1.20	1.32	1.66	1.52	1.45	1.33	1.00	0.79	0.44	0.20	0.16	0.60	0.83	0.65	0.36	0.59	0.57
											-	-	-	-	-	-					-
	1.63	4.23	5.23	8.22	24.6	24.8	15.2			-	10.5	14.3	21.4	22.3	25.1	27.7	-		22.0	23.1	23.4
vel:log(salrange)	E-11	E-11	E-10	E-12	3	6	7	5.77	4.54	4.82	7	9	7	7	9	5	7.90	8.05	9	0	4
	2.48	9.14	9.30	1.52				-	-	-	-	-	-	-	-	-	-	-			-
salmean:log(salrange)	E-13	E-13	E-12	E-13	0.50	0.50	0.17	0.16	0.20	0.44	0.50	0.55	0.64	0.60	0.59	0.60	0.24	0.01	0.22	0.29	0.53

	8.06	9.99	9.70	1.29	23.6	26.4	33.4	32.6	31.9	29.9	24.6	20.9	14.6	10.7		-	-	-	-	-16.0	19.3
vel:asin(em)	E-12	E-11	E-10	E-11	8	6	1	4	1	7	8	7	7	8	4.43	3.91	7.79	4.81	1.34	6	4
	1.17	2.81	2.81	3.54																-	
salmean:asin(em)	E-13	E-12	E-11	E-13	0.57	0.66	0.89	0.84	0.82	0.81	0.71	0.64	0.54	0.45	0.32	0.14	0.02	0.06	0.13	0.65	0.57
																-					
	3.96	3.84	3.36	4.09	10.9	11.3				-	-	-	-	-	-	10.0	-			-	
log(salrange):asin(em)	E-12	E-11	E-10	E-12	4	1	6.89	1.49	0.69	3.95	5.51	6.97	9.00	8.81	9.20	9	3.64	0.10	3.72	1.72	1.24
	-	-	-	-																	
	6.20	8.91	1.23	2.14	-	-	-											-	-	-	
vel:salmean:log(salrange)	E-13	E-13	E-11	E-13	0.73	0.72	0.26	0.11	0.13	0.43	0.59	0.68	0.89	0.90	0.97	1.00	0.33	0.26	0.81	0.90	1.07
	-	-	-	-																	
	3.64	2.79	2.96	4.06	-	-	-	-	-	-	-	-	-	-							-
vel:salmean:asin(em)	E-13	E-12	E-11	E-13	0.76	0.84	1.05	0.97	0.92	0.84	0.64	0.50	0.28	0.13	0.10	0.38	0.53	0.42	0.23	0.70	0.87
	-	-	-	-	-	-													-	-	
	1.08	4.25	4.18	5.47	15.6	15.8	-	-	-				13.6	14.2	16.0	17.6		-	14.0	10.9	
vel:log(salrange):asin(em)	E-11	E-11	E-10	E-12	8	3	9.72	3.68	2.89	3.07	6.73	9.16	7	4	3	6	5.03	5.12	6	6	4.54
	-	-	-	-																	
salmean:log(salrange):asin(e	1.57	8.74	8.42	1.05	-	-	-												-	-	
m)	E-13	E-13	E-12	E-13	0.32	0.32	0.11	0.10	0.12	0.28	0.32	0.35	0.41	0.38	0.38	0.38	0.15	0.01	0.14	0.03	0.03
vel:salmean:log(salrange):asi	4.22	9.48	1.03	1.43				-	-	-	-	-	-	-	-	-	-				-
n(em)	E-13	E-13	E-11	E-13	0.46	0.46	0.17	0.07	0.08	0.28	0.37	0.43	0.57	0.57	0.62	0.64	0.21	0.16	0.52	0.46	0.30
	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=	tau=
	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.54	0.59	0.64	0.69	0.74	0.79	0.84	0.89	0.94	0.99
	0	9	8	7	6	5	4	3	2	1	0	9	8	7	6	5	4	3	2	1	0

	4.05	9.64	1.04	1.56	27.6	32.1	43.8	43.7	44.4	44.4	41.5	39.0	35.1	32.2	27.1	19.6	15.2	17.4	20.2	24.2
(Intercept)	E-12	E-11	E-09	E-11	1	0	0	5	3	9	1	1	8	0	9	6	0	0	9	6.83	2
	-	-	-	-	-	-	-	-	-	-	-	-	-	-							-
	1.26	1.08	1.23	1.90	37.2	41.5	52.4	51.2	50.1	47.0	38.7	32.9	23.0	16.9	-		12.2				14.4
vel	E-11	E-10	E-09	E-11	0	6	8	7	3	7	7	4	4	3	6.95	6.14	3	7.56	2.11	9.35	2
	-	-	-	-																	
	2.13	3.11	3.32	5.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
salmean	E-13	E-12	E-11	E-13	0.90	1.03	1.40	1.31	1.30	1.27	1.11	1.00	0.85	0.71	0.50	0.22	0.03	0.09	0.20	0.30	0.52

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